

Fig.1.

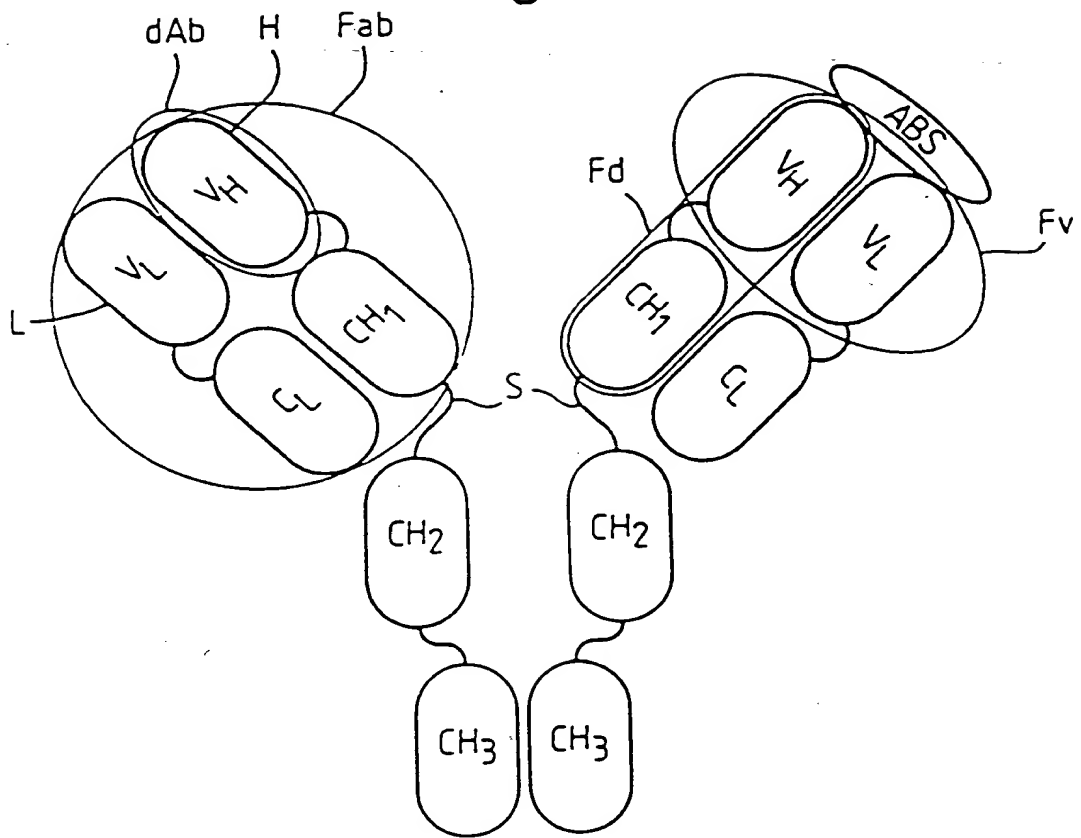


Fig.2 (i).

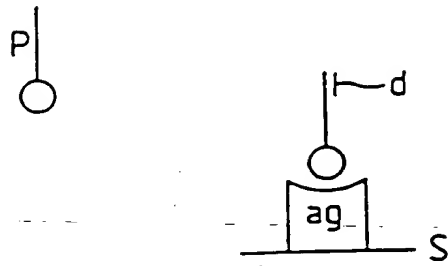


Fig.2 (ii).

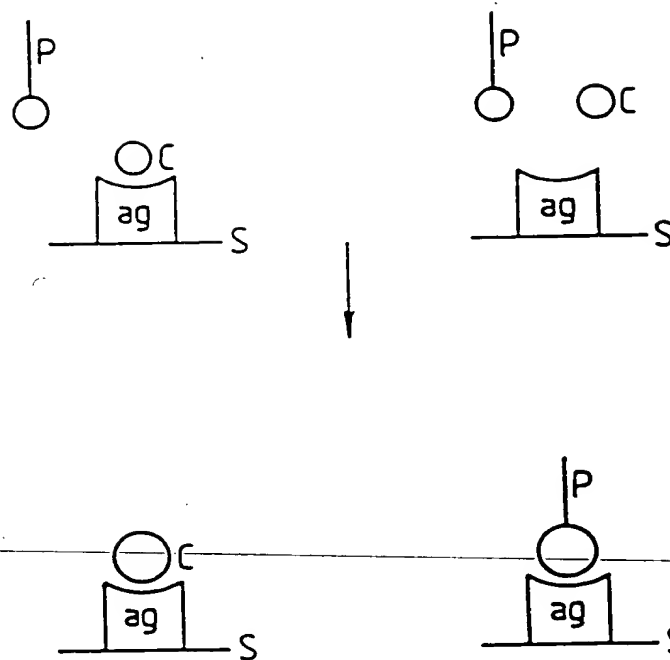
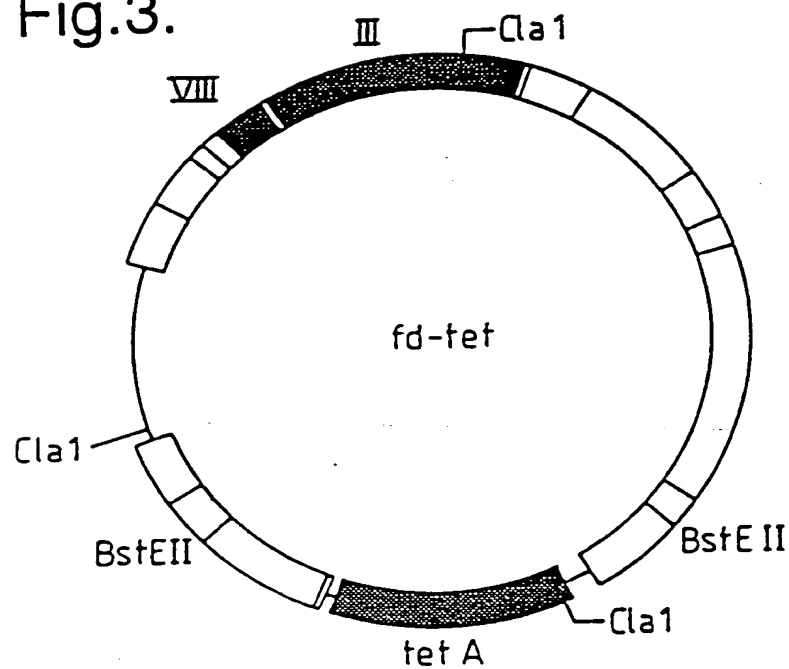


Fig.3.



fd - tet

~

cleave with BstEII

~

fill in with Klenow

~

re-ligate

↓

FDT6Bst

~

in vitro mutagenesis (oligo 1)

↓

FDTs/Bs

~

in vitro mutagenesis (oligo 2)

↓

FDTs/Xh

Oligo 1

(1653)
ACA ACT TTC AAC AGT TGA GGA GAC GGT GAC CGT AAG CTT CTG CAG TTG GAC CTG AGC (SEQ ID NO. 177)
GGA GTG AGA ATA (1620)

Oligo 2

(1653)
ACA ACT TTC AAC AGT TTC CCG TTT GAT CTC GAG CTC CTG CAG TTG GAC CTG (SEQ ID NO. 178)
(1704)
GTC GTC TTT CCA GAC GTT AGT (SEQ ID NO. 179)

Oligo 3

Fig.4 (i).

GENE III

GENE III

SIGNAL
CLEAVAGE SITE

(1624)
A TCT CAC TCC GCT

(1650)
GAA ACT GTT GAA AGT (SEQ ID NO. 180)

Q V Q L Q (SEQ ID NO. 1) V T V S S (SEQ ID NO. 2)

B TCT CAC TCC GCT CAG GTC CAA CTG CAG AAG CTT ACG GTC ACC GTC TCC TCA ACT GTT GAA AGT (SEQ ID NO. 181)
PstI BstEII

Q V Q L Q (SEQ ID NO. 1) L E I K R (SEQ ID NO. 3)

C TCT CAC TCC GCT CAG GTC CAA CTG CAG GAG CTC GAG ATC AAA CGG GAA ACT GTT GAA AGT (SEQ ID NO. 182)
PstI XhoI

Fig.4 (ii).

Fig.5.

rbs
M K Y L L P T A A
GCATGCAAATTCTATTTCAAGGAGACAGTCATAATGAAATACCTATTGCCTACGGCAGCC
10
20
30
40
50
60

SphI pelB leader

A G L L L L A A O P A M A Q V Q L Q E S

GCTGGATTGTTATTACTGCTGCCCAACCGATGGCCCAGGTGCAGCTGCAGGAGTCA

70 80 90 100 110 120

PstI

G P G L V A P S Q S L S I T C T V S G F
GGACCTGGCCTGGTGGCGCCCTCAGAGCCTGTCCATCATGCACCGTCTCAGGGTTC
130 140 150 160 170 180

S L T G Y G V N W V R Q P P G K G L E W
TCATTAACCGGCTATGGTGTAACCTGGGTTCCGCCAGCCTCCAGGAAAGGGTCTGGAGTGG
190 200 210 220 230 240

VHD1.3

L G M I W G D G N T D Y N S A L K S R L

CTGGGAATGATTTGGGGTGATGGAACACAGACTATAATTTCAGCTCTCAAATCCAGACTG

250 260 270 280 290 300

S I S K D N S K S Q V F L K M N S L H T
AGCATCAGCAAGGACAACCTCCAAGAGCCAGTTTTCTTAAAAATGAACAGTCTGCACACT
310 320 330 340 350 360

D D T A R Y Y C A R E R D Y R L D Y W G
GATGACACAGCCAGGTA C T A C T G T G C C A G A G A G A G A T T A T A G G C T T G A C T A C T G G G G C
370 380 390 400 410 420

Q G T T V T V S S G G G G S G G G S G
 CAAGGCACCACGGTCACGGTCTCTCTCAggtggaggcggttcaggcggaggtggctctggc
 430 440 450 460 470 480
 BstEII

G G G S D I E L T Q S P A S L S A S V G
 ggtggcggaatcgGACATCGAGCTCACTCAGTCTCCAGCCTCCCTTTCTGCGTCTGTGGGA
 490 500 510 520 530 540
 SacI

Fig.5 (Cont).

E T V T I T C R A S G N I H N Y L A W Y
GAAACTGTCACCATCACATGTCGAGCAAGTGGGAATATTACAAATTATTTAGCATGGTAT
550 560 570 580 590 600

Q Q K Q G K S P Q L L V Y Y T T T L A D
CAGCAGAAACAGGGAAAATCTCCTCAGCTCCTGGTCTATTATACAACAACCTTAGCAGAT
610 620 630 640 650 660

VKD1.3

G V P S R F S G S G S G T Q Y S L K I N
GGTGTGCCATCAAGGTTTCAGTGGCAGTGGATCAGGAACACAATATTCTCTCAAGATCAAC
670 680 690 700 710 720

S L Q P E D F G S Y Y C Q H F W S T P R
AGCCTGCAACCTGAAGATTTTGGGAGTTTACTGTCAACATTTTGGAGTACTCCTCGG
730 740 750 760 770 780

Myc Tag (TAG1)

T F G G G T K L E I K R E O K L I S E E
ACGTTCCGGTGGAGGGACCAAGCTCGAGATCAAACGGGAACAAAACTCATCTCAGAAGAG
790 800 810 820 830 840

XhoI

D L N * * (SEQ ID NO. 183)

GATCTGAATTAATAATGATCAAACGGTAATAAGGATCCAGCTCGAATTC (SEQ ID NO. 184)
850 860 870 880

EcoRI

Fig.6.

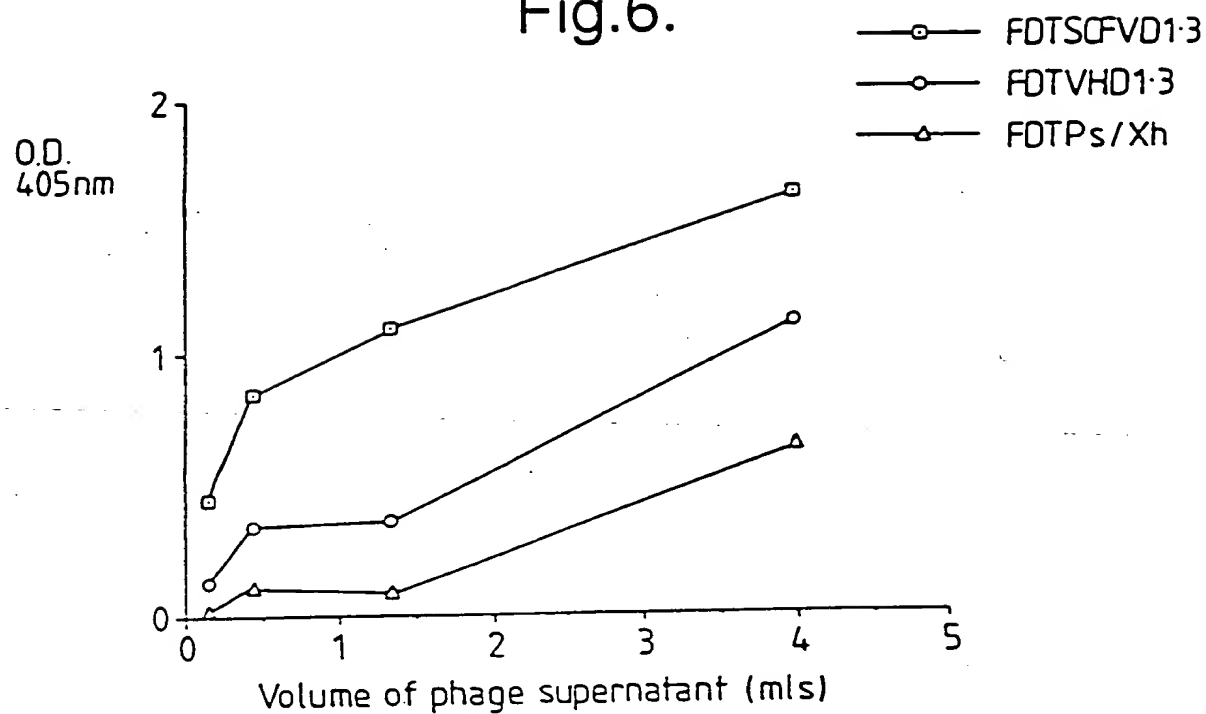


Fig.7.

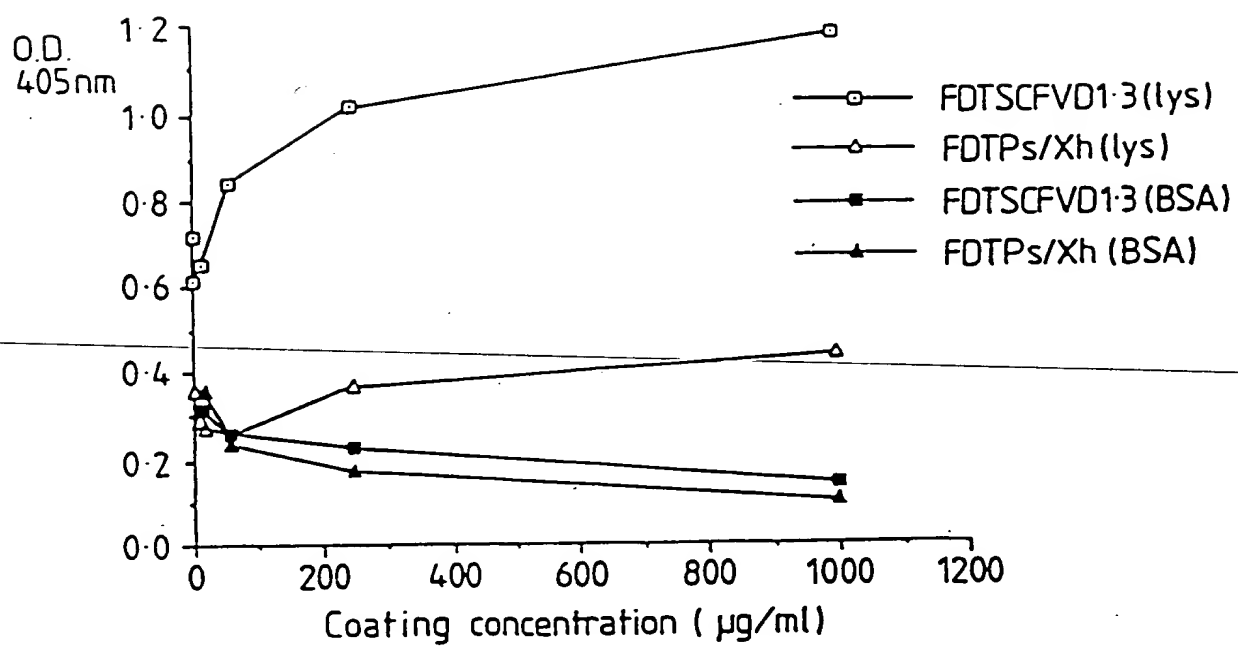


Fig.8.

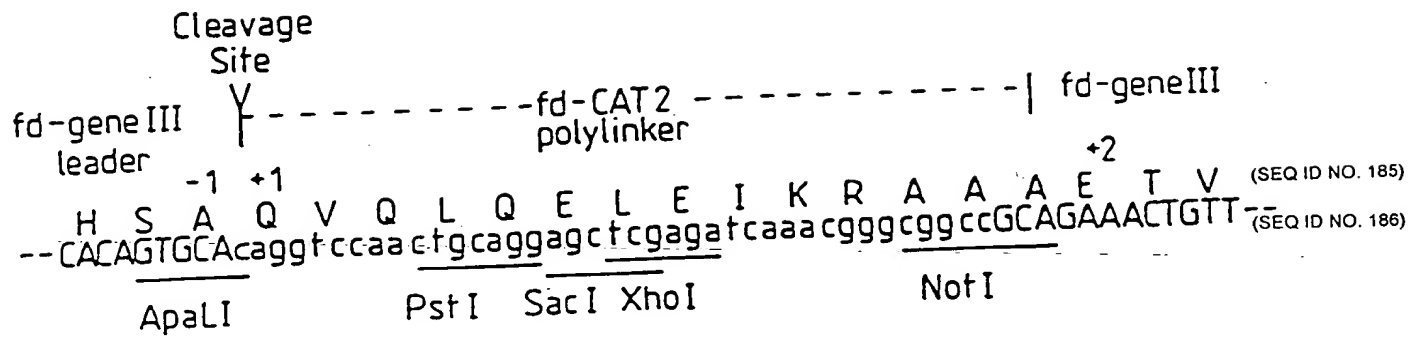


Fig.9.

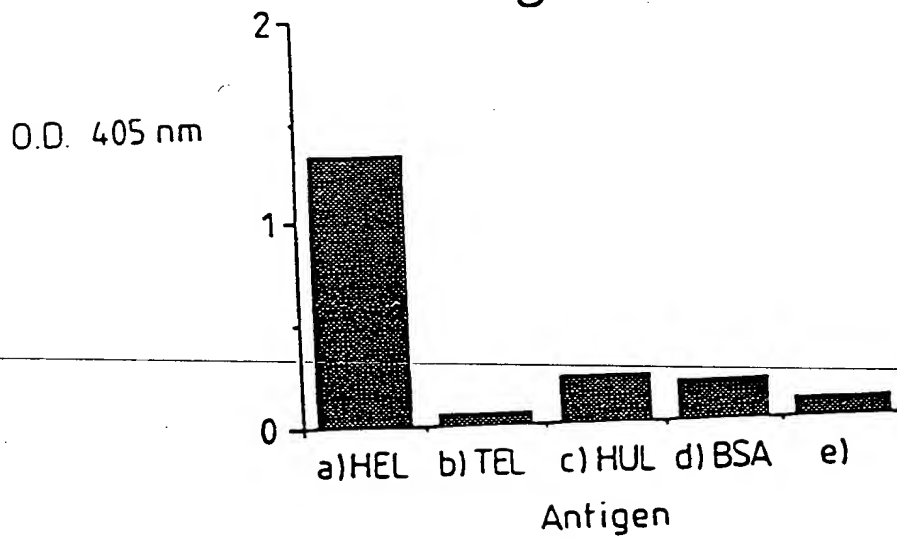


Fig.10.

M K Y L L P T A A
GCATGCAAATTCTATTTC AAGGAGACAGTCATAATGAAATACCTATTGCTACGGCAGCC
10 20 30 40 50 60

A G L L L L A A Q P A M A Q V Q L Q E S
GCTGGATTGTTATTACTGCTGCCCCAACCAGCGATGGCCCCAGGTGCAGCTGCAGGAGTCA
70 80 90 100 110 120

G P G L V A P S Q S L S I T C T V S G F
GGACCTGGGCTGGTGGCGCCCTCACAGCGCTGTCCATCACATGCACCGTCTCAGGGTTC
130 140 150 160 170 180

S L T G Y G V N W V R Q P P G K G L E W
TCATTACCGGCTATGGTGTAACTGGGTTCGCCAGCGCTCCAGGAAAGGGTCTGGAGTGG
190 200 210 220 230 240

L G M I W G D G N T D Y N S A L K S R L
CTGGGAATGATTGTTGGGGTIGATGGAACACAGACTATAATTACGCTCTCAAATCCAGACTG
250 260 270 280 290 300

S I S K D N S K S Q V F L K M N S L H T
AGCATCAGCAAGGACAACCTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCACACT
310 320 330 340 350 360

D D T A R Y Y C A R E R D Y R L D Y W G
GATGACACAGCCAGGTACTACTGTGCCAGAGAGAGAGATTATAGGCTTGACTACTGGGGC
370 380 390 400 410 420

Q G T T V T V S S A S T K G P S V F P L
CAAGGCACCAAGGTACCGTCTCCTCAGCGCTCCACCAAGGGCCCATGGGTCTTCCCCCTG
430 440 450 460 470 480

A P S S K S T S G G T A A L G C L V K D
GCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGGGGCCCTGGGCTGGTCAAGGAC
490 500 510 520 530 540

Fig.10 (Cont 1).

Y F P E P V T V S W N S G A L T S G V H
TACTTCCCCGAACCGGTGACGGTGTGGTGGAACTCAGGCGCCCTGACCAGCGGGGTGCAC
550 560 570 580 590 600

T F P A V L Q S S G L Y S L S S V V T V
ACCTTCCCGGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTG
610 620 630 640 650 660

P S S S L G T Q T Y I C N V N H K P S N
CCCTCCAGCAGCTTGGGCAACCAGACCTACATCTGCAACGTGAATCACAAGCCCAAGCAAC
670 680 690 700 710 720

T K V D K K V E P K S S * * (SEQ ID NO. 187)
ACCAAGGTGACACAAGAAAGTTCAGCCCAATCTTCATAATAACCCGGGAGCTTGCATGCA
730 740 750 760 770 780

M K Y L L P T A A A G L
AATTCTATTTCAGGAGACAGTCTAATGAATAACCTATTGGCTACGGCAGCCGCTGGAT
790 800 810 820 830 840

L L L A A Q P A M A D I E L T Q S P A S
TGTTATTACTGGCTGGCCCAACCAGCGATGGCCGACATCGAGCTCACCAGTCTCCAGCCT
850 860 870 880 890 900

L S A S V G E T V T I T C R A S G N I H
CCCTTCTCGGCTCTGTGGGAGAAACGTGCACCATCAGATGTGGAGCAAGTGGGAATATT
910 920 930 940 950 960

N Y L A W Y Q Q K Q G K S P Q L L V Y Y
ACAATTATTTAGCATGGTATCAGCAGAAACAGGGAAATCTCCTCAGCTCCTGGTCTATT
970 980 990 1000 1010 1020

Fig.10 (Cont 2).

T T T L A D G V P S R F S G S G S G T Q
ATACAACAACCTTAGCAGATGGTGTGCCATCAAGGTTTCAGTGGCAGTGGATCAGGAACAC
1030 1040 1050 1060 1070 1080

Y S L K I N S L Q P E D F G S Y Y C Q H
AATATTCTCTCAAGATCAACAGCCTGCGAGCCTGAAGATTTTGGGAGTTATTACTGTCAAC
1090 1100 1110 1120 1130 1140

F W S T P R T F G G G T K L E I K R T V
ATTTTGGAGTACTCCTCGGACGTTGGTGGAGGCACCAAGCTCGAGATCAAAACGGACTG
1150 1160 1170 1180 1190 1200

A A P S V F I F P P S D E Q L K S G T A
TGGCTGCAACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTG
1210 1220 1230 1240 1250 1260

S V V C L L N N F Y P R E A K V Q W K V
CCTCTGTGTGTGTGCGCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGAAGG
1270 1280 1290 1300 1310 1320

D N A L Q S G N S Q E S V T E Q D S K D
TGGATAACGCCCTCCCAATCGGGTAACTCCCAGGAGAGTGTCAACAGAGCAGGACAGCAAGG
1330 1340 1350 1360 1370 1380

S T Y S L S S T L T L S K A D Y E K H K
ACAGCACCTACAGCCTCAGCAGCAACCTGACGCTGAGCAAAGCAGACTACGAGAAACACA
1390 1400 1410 1420 1430 1440

V Y A C E V T H Q G L S S P V T K S F N
AAGTCTAAGCCTGCGAAGTCAACCATCAGGGCCTGAGCTCGCCCGTCAAAAGAGCTTCA
1450 1460 1470 1480 1490 1500

R G E S * * (SEQ ID NO. 188)
ACCGGGAGAGTCATAGTAAGAATTC (SEQ ID NO. 189)
1510 1520

Fig.10 (Cont 3).

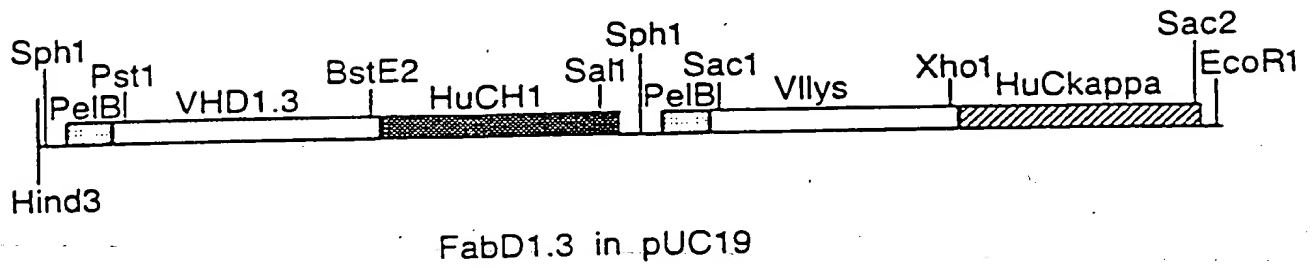


Fig.11.

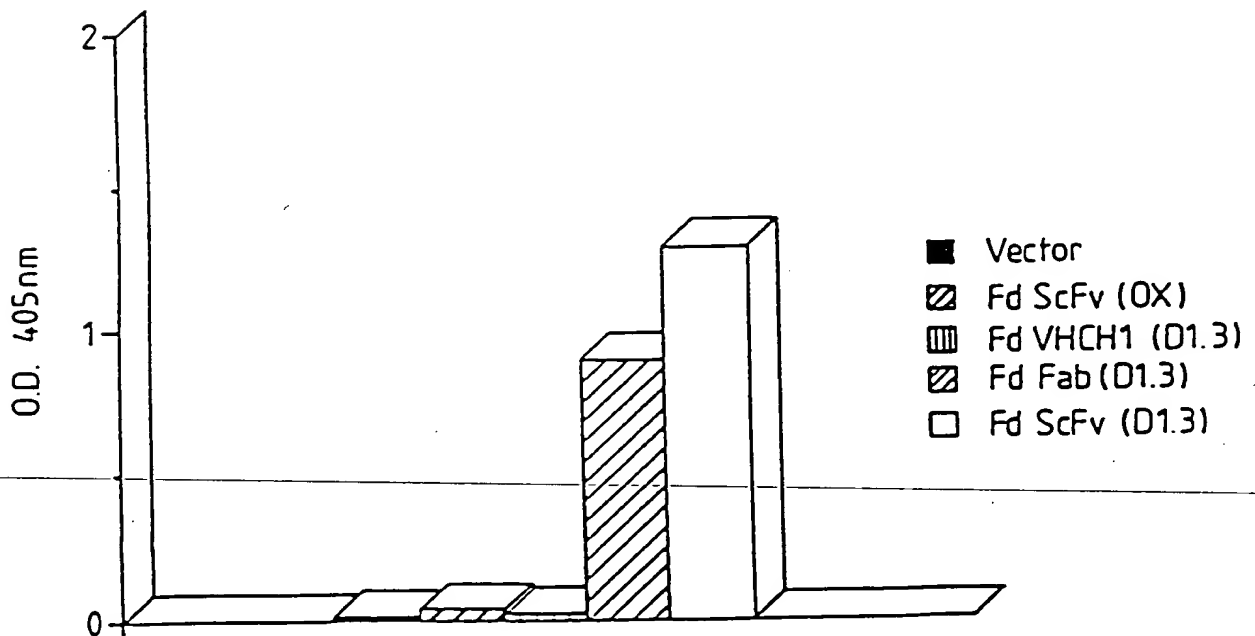


Fig.12a.

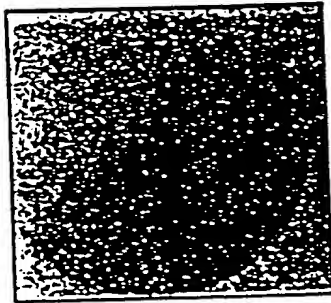


Fig.12b.

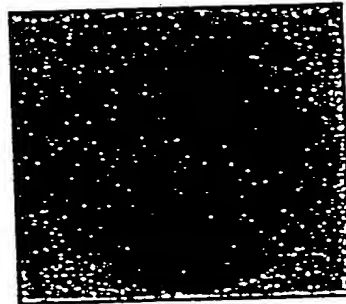


Fig.13.

Q V Q L Q E S G G G L V Q P G G
 CAG GTG CAG CTG CAG GAG TCA GGA GGA GGC TTG GTA CAG CCT GGG GGT
 PstI
 S L R L S C A T S G F T F S N Y
 TCT CTG AGA CTC TCC TGT GCA ACT TCT GGG TTC ACC TTC AGT AAT TAC
 Y M G W V R Q P P G K A L E W L
 TAC ATG GGC TGG GTC CGC CAG CCT CCA GGA AAG GCA CTT GAG TGG TTG
 G S V R N K V N G Y T T E Y S A
 GGT TCT GTT AGA AAC AAA GTT AAT GGT TAC ACA ACA GAG TAC AGT GCA
 S V K G R F T I S R D N F Q S I
 TCT GTG AAG GGG CGG TTC ACC ATC TCC AGA GAT AAT TTC CAA AGC ATC
 L Y L Q I N T L R T E D S A T Y
 CTC TAT CTT CAA ATA AAC ACC CTG AGA ACT GAG GAC AGT GCC ACT TAT
 Y C A R G Y D Y G A W F A Y W G
 TAC TGT GCA AGA GGC TAT GAT TAC GGG GCC TGG TTT GCT TAC TGG GGC
 Q G T L V T v s s g g g g s g g g g s
 CAA GGG ACC CTG GTC ACC gtc tcc tca ggtgggaggcggttcaggcgggagggtggctct
 BstEII
 g g g g s d i E L T Q T P L S L P V
 ggcggtggcggtcgggac atc GAG CTC ACC CAA ACT CCA CTC TCC CTG CCT GTC
 SacI
 S L G D Q A S I S C R S S Q S I
 AGT CTT GGA GAT CAA GCC TCC ATC TCT TGC AGA TCT AGT CAG AGC ATT
 V H S N G N T Y L E W Y L Q K P
 GTA CAT AGT AAT GGA AAC ACC TAT TTA GAA TGG TAC CTG CAG AAA CCA
 PstI
 G Q S P K L L I Y K V S N R F S
 GGC CAG TCT CCA AAG CTC CTG ATC TAC AAA GTT TCC AAC CGA TTT TCT
 G V P D R F S G S G S G T D F T
 GGG GTC CCA GAC AGG TTC AGT GGC AGT GGA TCG GGG ACA GAT TTC ACA
 L K I S R V E A E D L G V Y Y C
 CTC AAG ATC AGC AGA GTG GAG GCT GAG GAT CTG GGA GTT TAT TAC TGC
 F Q G S H V P Y T F G G G T K L
 TTT CAA GGT TCA CAT GTT CCG TAC ACG TTC GGA GGG GGG ACC AAG CTC
 E I K R
GAG ATC AAA CGG (SEQ ID NO. 190)
 XhoI (SEQ ID NO. 191)

Fig.14.

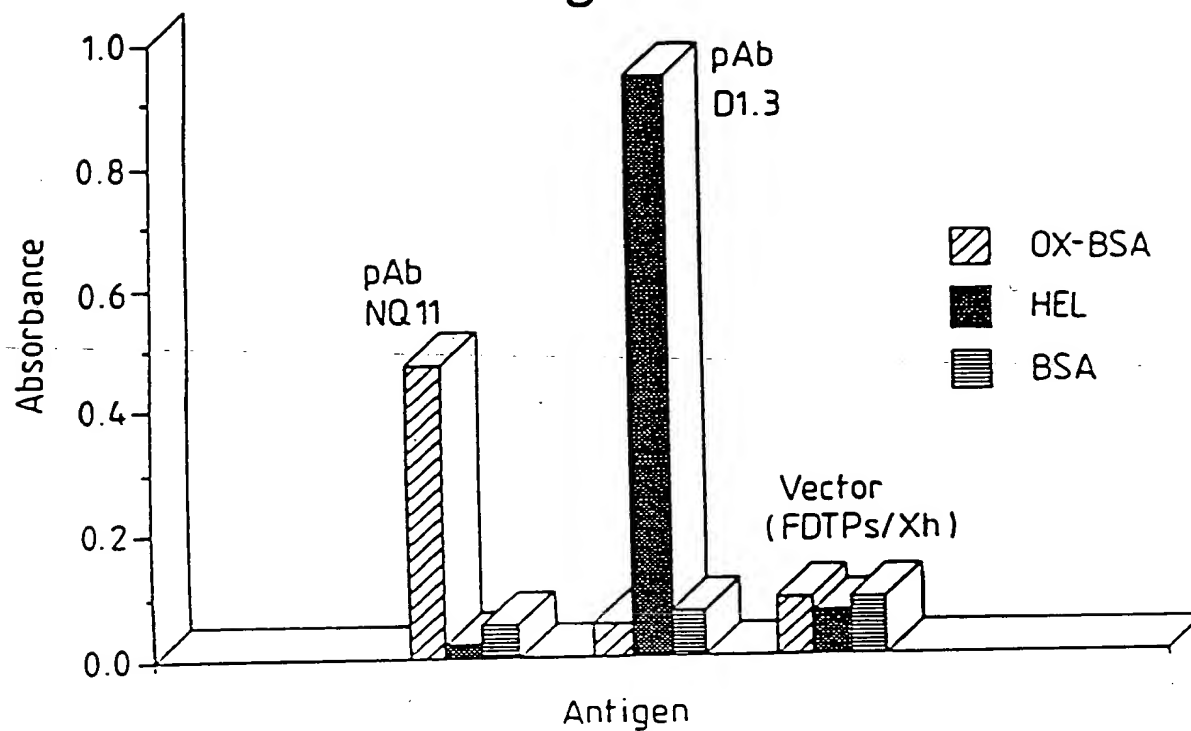


Fig.15.

5' END

TCT CAC AGT GCA CAA ACT GTT GAA CGG ACA CCA GAA ATG CCT GTT CTG (SEQ ID NO. 192)
 TCT CAC AGT GCA CAA ACT GTT GAA CGG ACA CCA GAA ATG CCT GTT CTG (SEQ ID NO. 193)
 ApaL1

3' END

K A A L G L K
 AAA GCC GCT CTG GGG CTG AAA GCG GCC GCA GAA ACT GTT GAA AGT etc.
 Not I

Fig.16 (i).

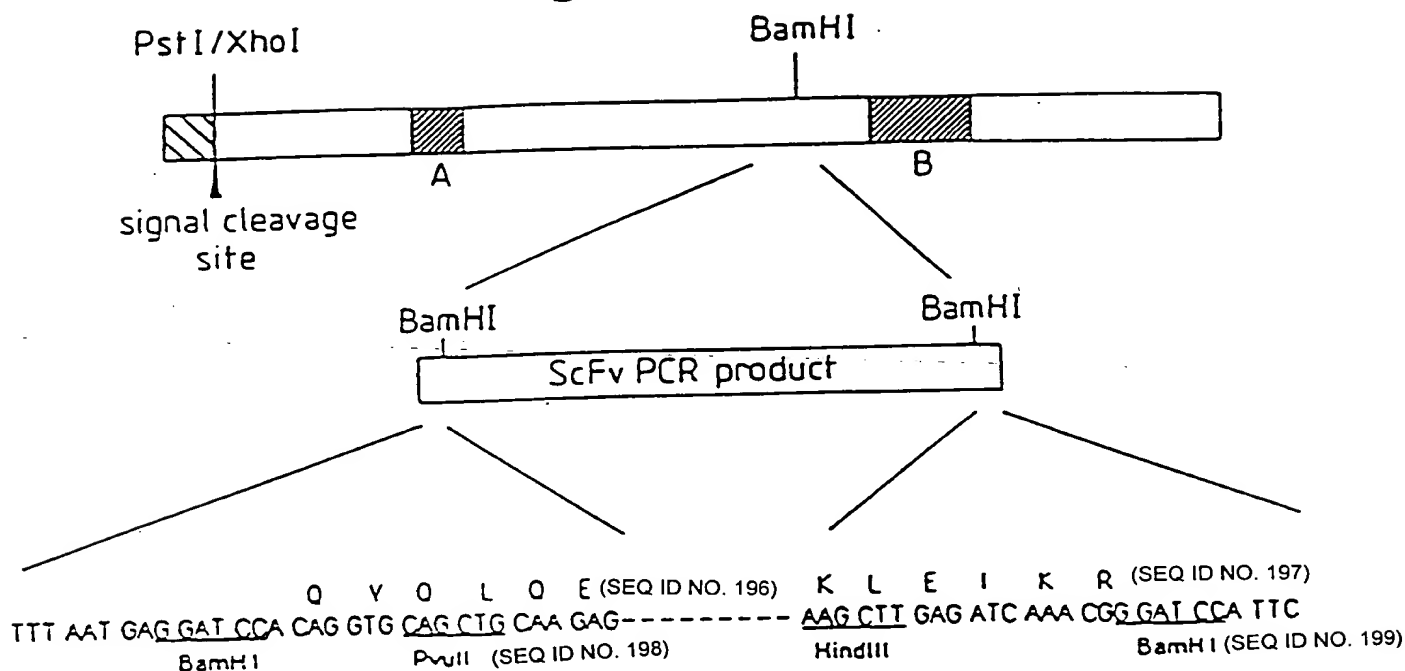


Fig.16 (ii).

A (1834) 5' GAG GGT GGT GGC TCT (SEQ ID NO. 200)
 - - -C - - (SEQ ID NO. 201)
 - - -C - - (SEQ ID NO. 202)
 - - -C - ACT 3'(1839) (SEQ ID NO. 203)

B (2284) 5' - GGC GGC GGC TCT (SEQ ID NO. 204)
 - GGT GGT GGT - (SEQ ID NO. 205)
 - - GGC GGC - (SEQ ID NO. 206)
 GAG - - GGC - (SEQ ID NO. 207)
 - - - GGT - (SEQ ID NO. 208)
 - - - GGC - (SEQ ID NO. 209)
 - - - GGT - (SEQ ID NO. 210)
 - - - GGC - 3'(2379) (SEQ ID NO. 211)

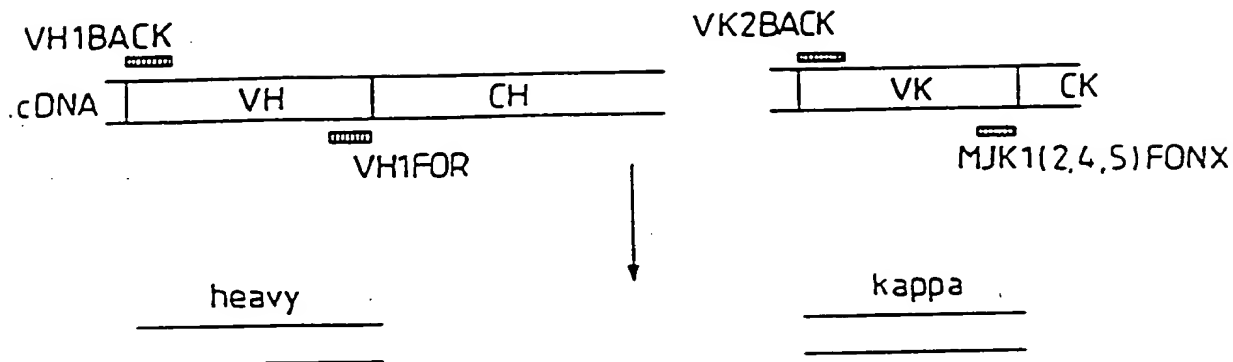
Reverse complement of mutagenic
 oligo G3Bamlink

5' GAG GGT GGC GGA TCC (SEQ ID NO. 212)

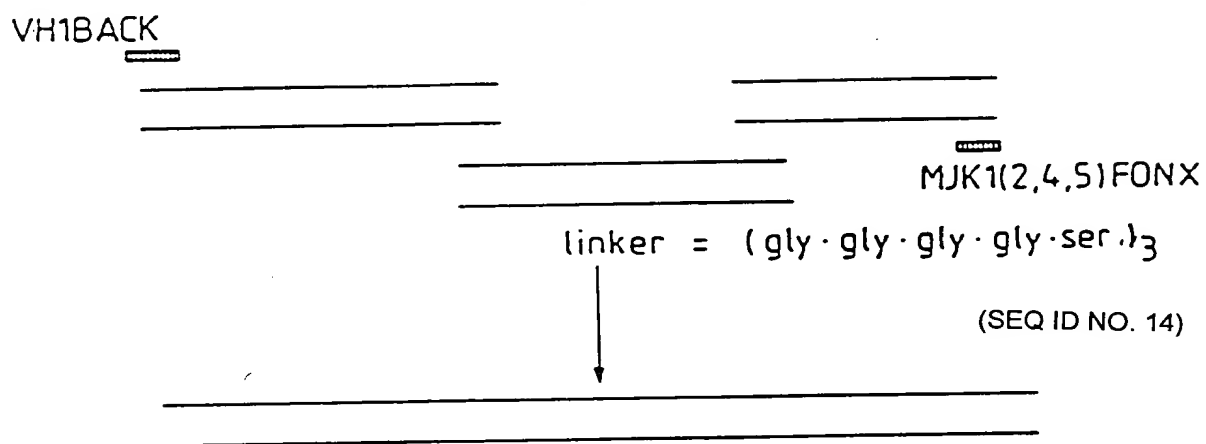
GAG GGT GGC GG 3' (SEQ ID NO. 213)

Fig.17.

1) PRIMARY PCR



2) ASSEMBLY PCR



3) ADDING RESTRICTION SITES

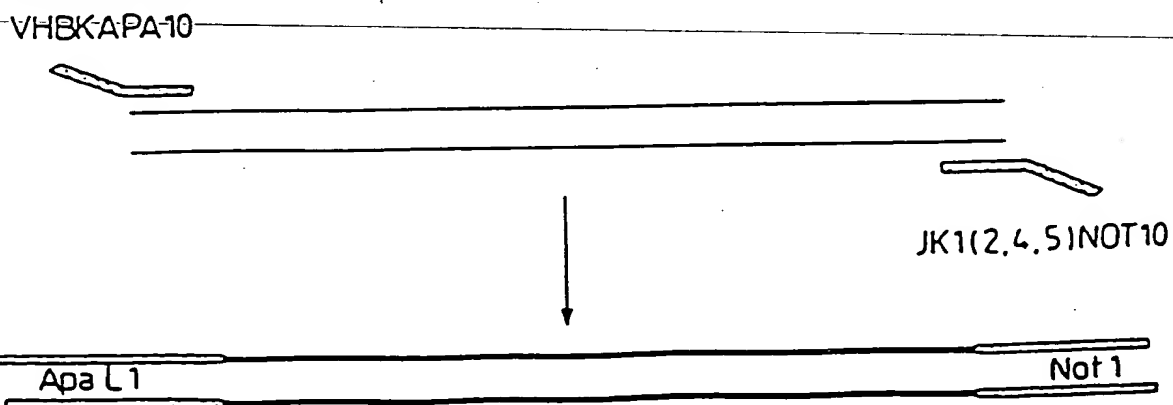


Fig.18.

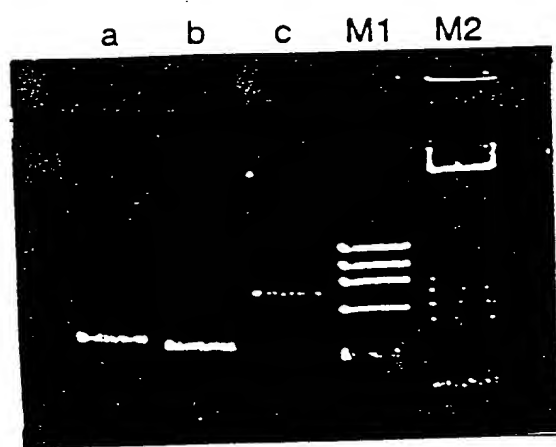


Fig.19.

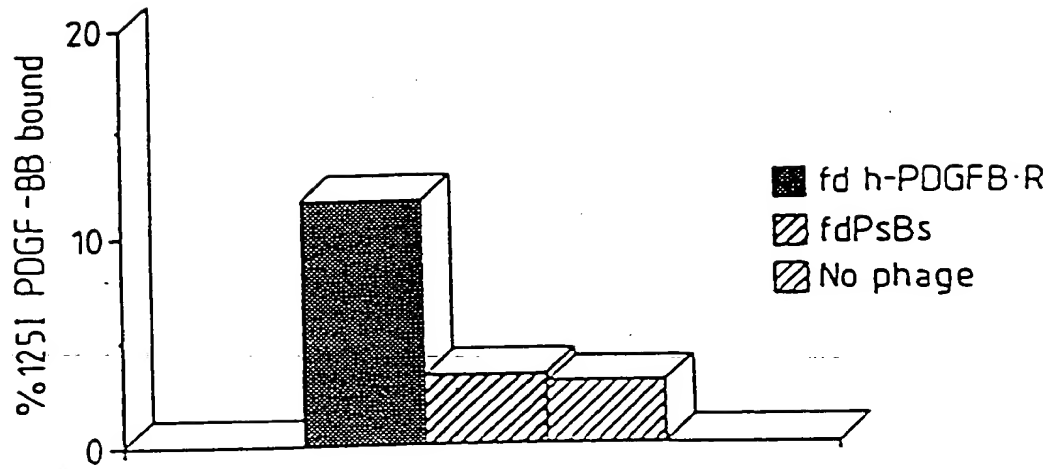


Fig.20.

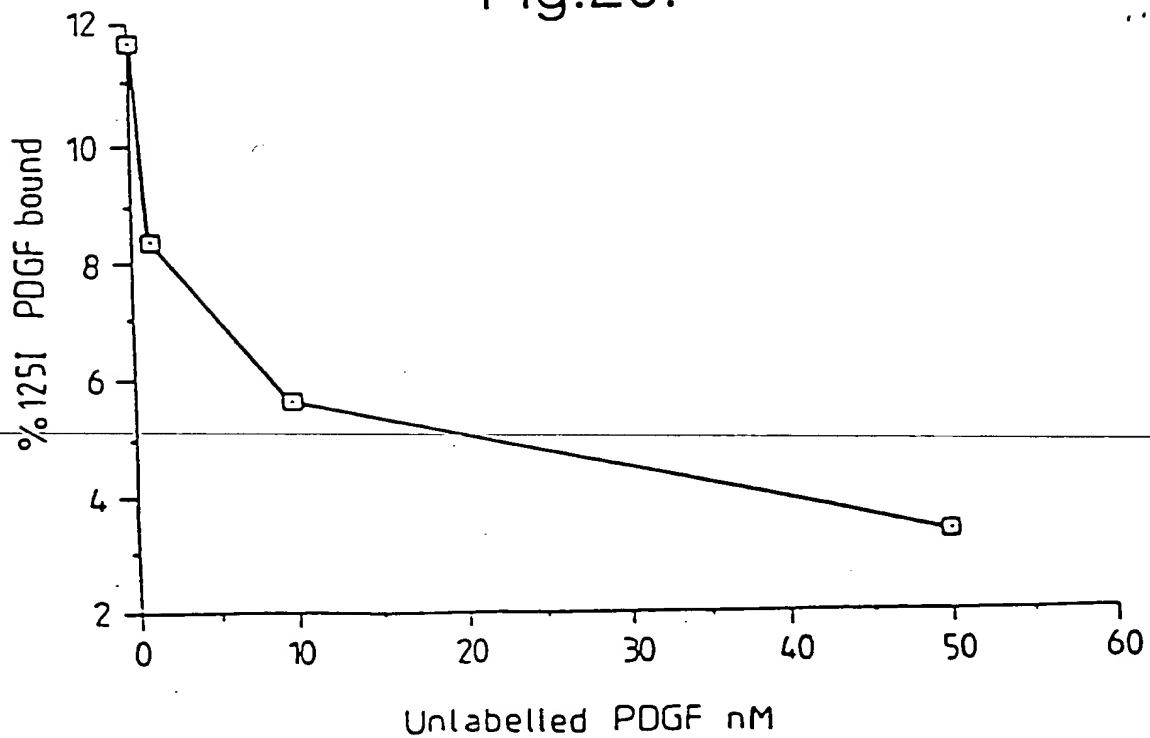


Fig.21.

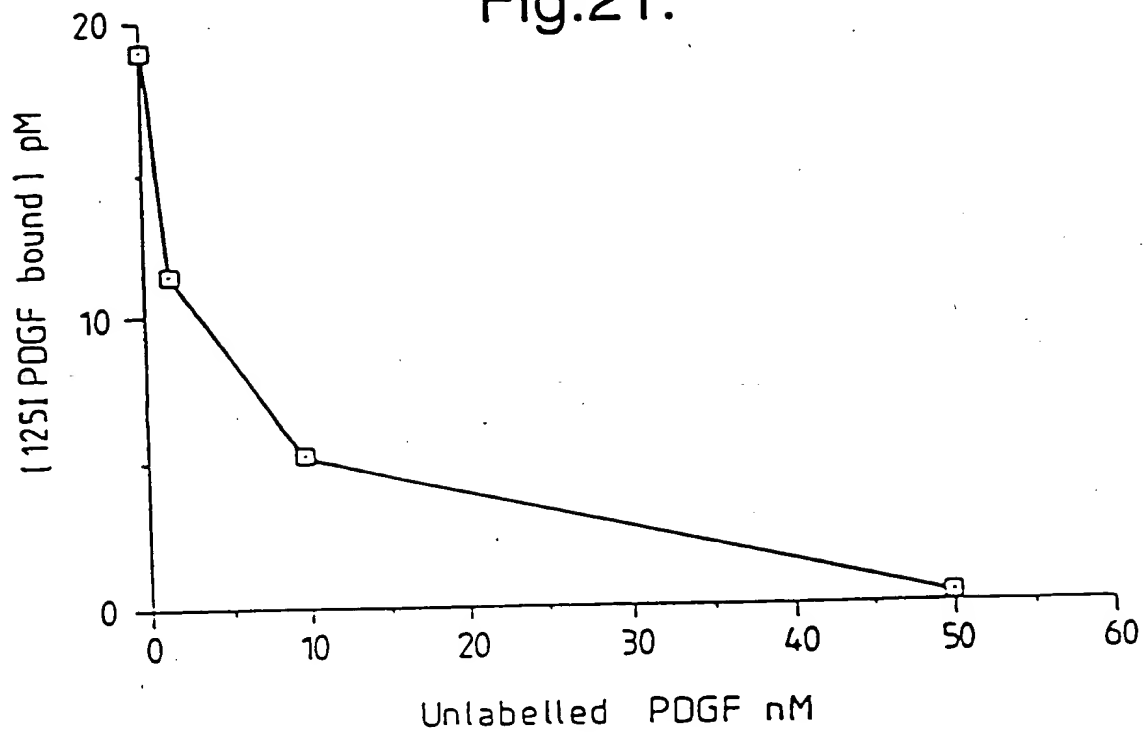


Fig.22.

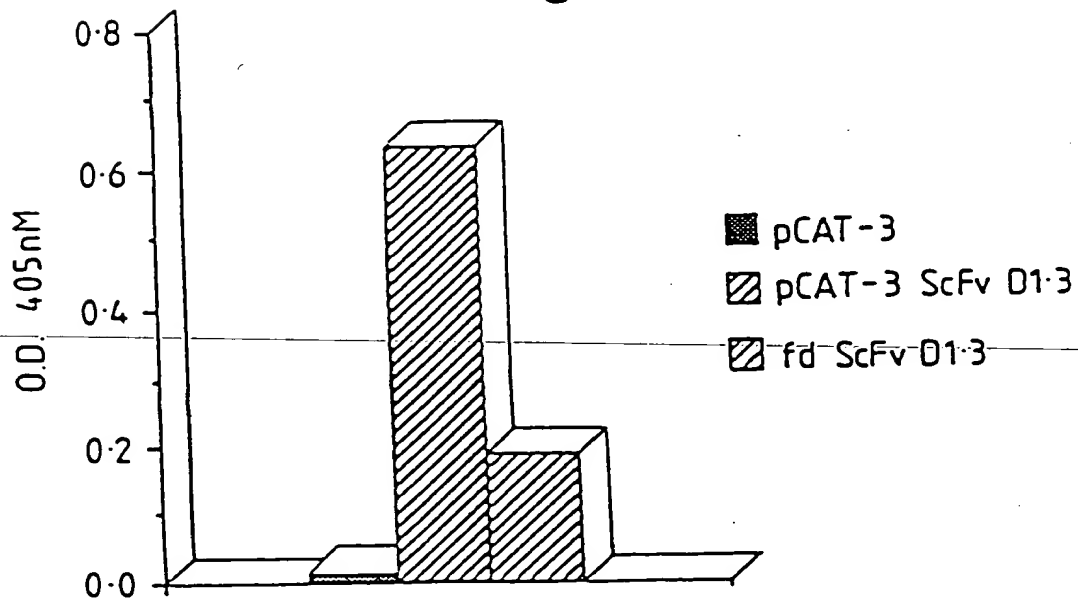


Fig.23(i)

d
M

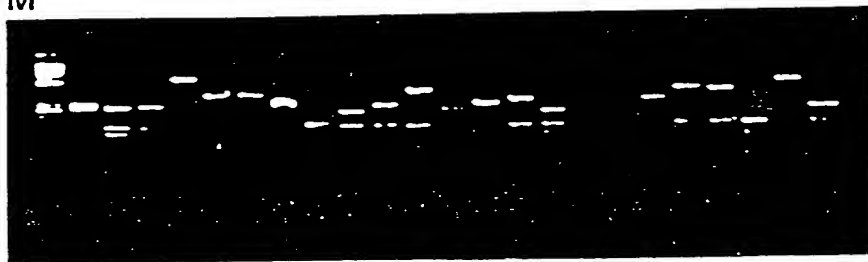


Fig.23(ii)

M



VH sequences

Fig.24.

from combinatorial library:

	CDR1	CDR2	CDR3	
A	QVQLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
B	QVRLQSGDAELAKPGASVYKSCKASGTTTT	RDTHMI	MLKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
C	QVQLQSGDAELVYKPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
D	QVQLQSGDAELVYKPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
E	QVQLQSGDAELVYKPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
F	QVQLQSGDAELVYKPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
G	QVQLQSGDAELVYKPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
H	QVQLQSGDAELVYKPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD

from hierarchical library VH-sep & Vc-d:

	CDR1	CDR2	CDR3	
I	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
J	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
K	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
L	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
M	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
N	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
O	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
P	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
Q	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
R	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
S	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
T	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
U	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
V	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
W	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
X	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
Y	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD
Z	QVRLQSGDAELARPGASVYKSCKASGTTTT	STTHMI	MYKQRPQCGCLEMIG	YINPSSGCTYTHNQKFKD

Fig.24 (Cont).

Vx sequences

from combinatorial library:

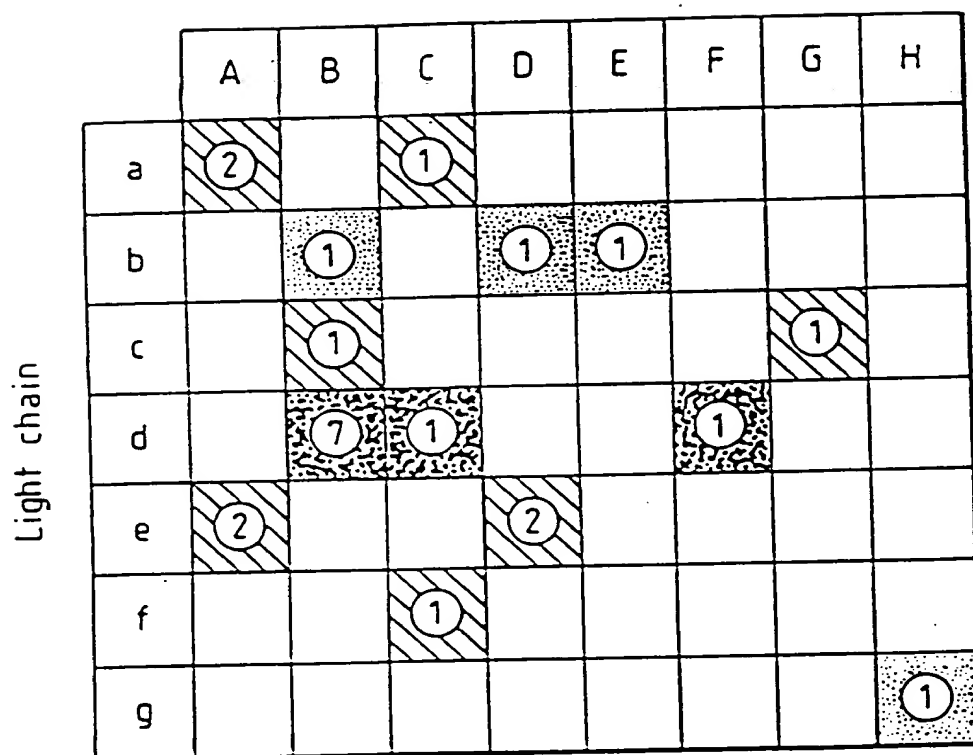
	CDR1	CDR2	CDR3			
a	DIELTQSPSSLSASLGERVSLTC	RASQELSGTSL	WLOQRPGDSIKRLIY	AASTLSS	GVVPARFSGSRSGSGLTSLTSSSEDAEDAAATTC	LOYASYPT
b	DIELTQSPAIHNSASPGKVTTC	RASSSV66SYLH	MYQOKPGCTSPKRLIY	BTSHLAS	GVVPARFSGSGSGTSLTSSVEAEDAAATTC	QOYSGYPLT
c	DIELTQSPPTTHAASPGKVTTC	SASSS16SNTLH	MYQOKPGFSPKRLIY	RTSHLAS	GVVPARFSGSGSGTSLTIGTHEAEDVATTC	QOQSS1PLT
d	DIELTQSPPTTHAASPGKVTTC	SASSS16SNTLH	MYQOKPGFSPKLLIS	RTSHLAS	GVVPARFSGSGSGTSLTIGTHEAEDVATTC	QOQST1PPT
e	DIELTQSPAIHNSASPGKVTTC	SASSSVNTTH	MYQOKPGCTSPKRLIY	STSHLAS	GVVPARFSGSGSGTSLTSSHEAEDAAATTC	QOQSS1PPT
f	DIELTQSPAIHNSASPGKVTTC	SASSSVNTTH	MYQOKSGTSPKRLIY	DTSHLAS	GVVPARFSGSGSGTSLTSSHEAEDAAATTC	QOQSS1PPT
g	DIELTQSPAIHNSASPGKVTTC	SASSS1NTTH	MYQOKPGASPKRLIY	DTSHLAS	GVVPARFSGSGSGTSLTSSHEAEDAAATTC	QOQSS1PPT

from hierarchical library VII-B x Vx-rep:

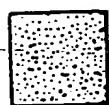
	CDR1	CDR2	CDR3			
h	DIELTQSPAIHNSASPGKVTTC	SASSSVNTTH	MYQOKSGTSPKRLIY	DTSHLAS	GVVPARFSGSGSGTSLTSSHEAEDAAATTC	QOQSS1PPT
i	DIELTQSPAIHNSASPGKVTTC	SASSSVNTTH	MYQOKPGCTSPKRLIY	STSHLAS	GVVPARFSGSGSGTSLTSSHEAEDAAATTC	QOQSS1PPT
j	DIELTQSPPTTHAASPGKVTTC	SASSS1SNTLH	MYQOKPGFSPKRLIY	RTSHLAS	GVVPARFSGSGSGTSLTIGTHEAEDVATTC	QOQSS1PPT
k	DIELTQSPPTTHAASPGKVTTC	SASSS1SNTLH	MYQOKPGFSPKRLIY	RTSHLAS	GVVPARFSGSGSGTSLTIGTHEAEDVATTC	QOQSS1PPT
l	DIELTQSPPTTHAASPGKVTTC	SASSS1SNTLH	MYQOKPGFSPKRLIY	RTSHLAS	GVVPARFSGSGSGTSLTIGTHEAEDVATTC	QOQSS1PPT
m	DIELTQSPPTTHAASPGKVTTC	SASSS1SNTLH	MYQOKPGFSPKRLIY	RTSHLAS	GVVPARFSGSGSGTSLTIGTHEAEDVATTC	QOQSS1PPT
n	DIELTQSPPTTHAASPGKVTTC	SASSS1SNTLH	MYQOKPGFSPKRLIY	RTSHLAS	GVVPARFSGSGSGTSLTIGTHEAEDVATTC	QOQSS1PPT
o	DIELTQSPAIHNSASPGKVTTC	SASSSVNTTH	MYQOKPGFSPKRLIY	DTSHLAS	GVVPARFSGSGSGTSLTSSHEAEDAAATTC	QOQSS1PPT
p	DIELTQSPAIHNSASPGKVTTC	SASSSVNTTH	MYQOKPGFSPKRLIY	DTSHLAS	GVVPARFSGSGSGTSLTSSHEAEDAAATTC	QOQSS1PPT
q	DIELTQSPAIHNSASPGKVTTC	SASSSVNTTH	MYQOKPGFSPKRLIY	DTSHLAS	GVVPARFSGSGSGTSLTSSHEAEDAAATTC	QOQSS1PPT
r	DIELTQSPAIHNSASPGKVTTC	SASSSVNTTH	MYQOKPGFSPKRLIY	DTSHLAS	GVVPARFSGSGSGTSLTSSHEAEDAAATTC	QOQSS1PPT
s	DIELTQSPAIHNSASPGKVTTC	SASSSVNTTH	MYQOKPGFSPKRLIY	DTSHLAS	GVVPARFSGSGSGTSLTSSHEAEDAAATTC	QOQSS1PPT
t	DIELTQSPAIHNSASPGKVTTC	SASSSVNTTH	MYQOKPGFSPKRLIY	DTSHLAS	GVVPARFSGSGSGTSLTSSHEAEDAAATTC	QOQSS1PPT
u	DIELTQSPAIHNSASPGKVTTC	SASSSVNTTH	MYQOKPGFSPKRLIY	DTSHLAS	GVVPARFSGSGSGTSLTSSHEAEDAAATTC	QOQSS1PPT
v	DIELTQSPAIHNSASPGKVTTC	SASSSVNTTH	MYQOKPGFSPKRLIY	DTSHLAS	GVVPARFSGSGSGTSLTSSHEAEDAAATTC	QOQSS1PPT
w	DIELTQSPAIHNSASPGKVTTC	SASSSVNTTH	MYQOKPGFSPKRLIY	DTSHLAS	GVVPARFSGSGSGTSLTSSHEAEDAAATTC	QOQSS1PPT

Fig.25.

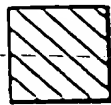
HEAVY CHAIN



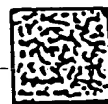
OD_{405nm} in ELISA



0.2-0.9



0.9-2.0



>2.0

Fig.26(a).

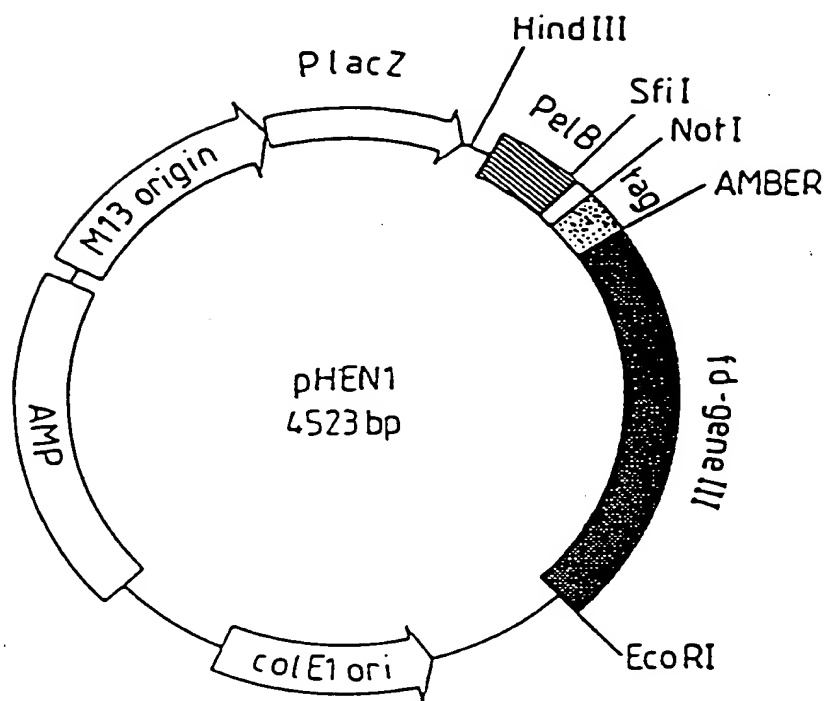


Fig.26(b).

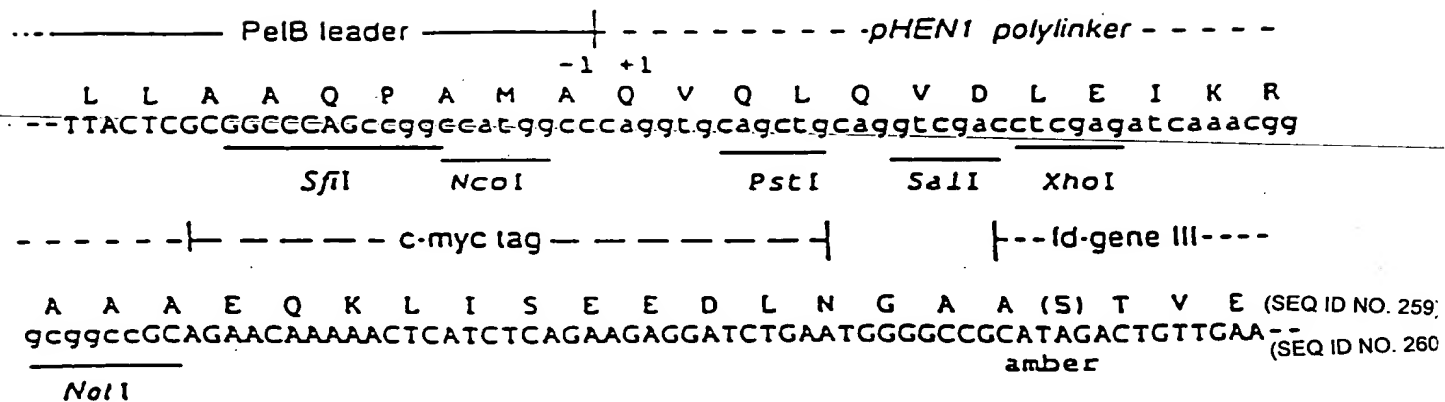


Fig.27.

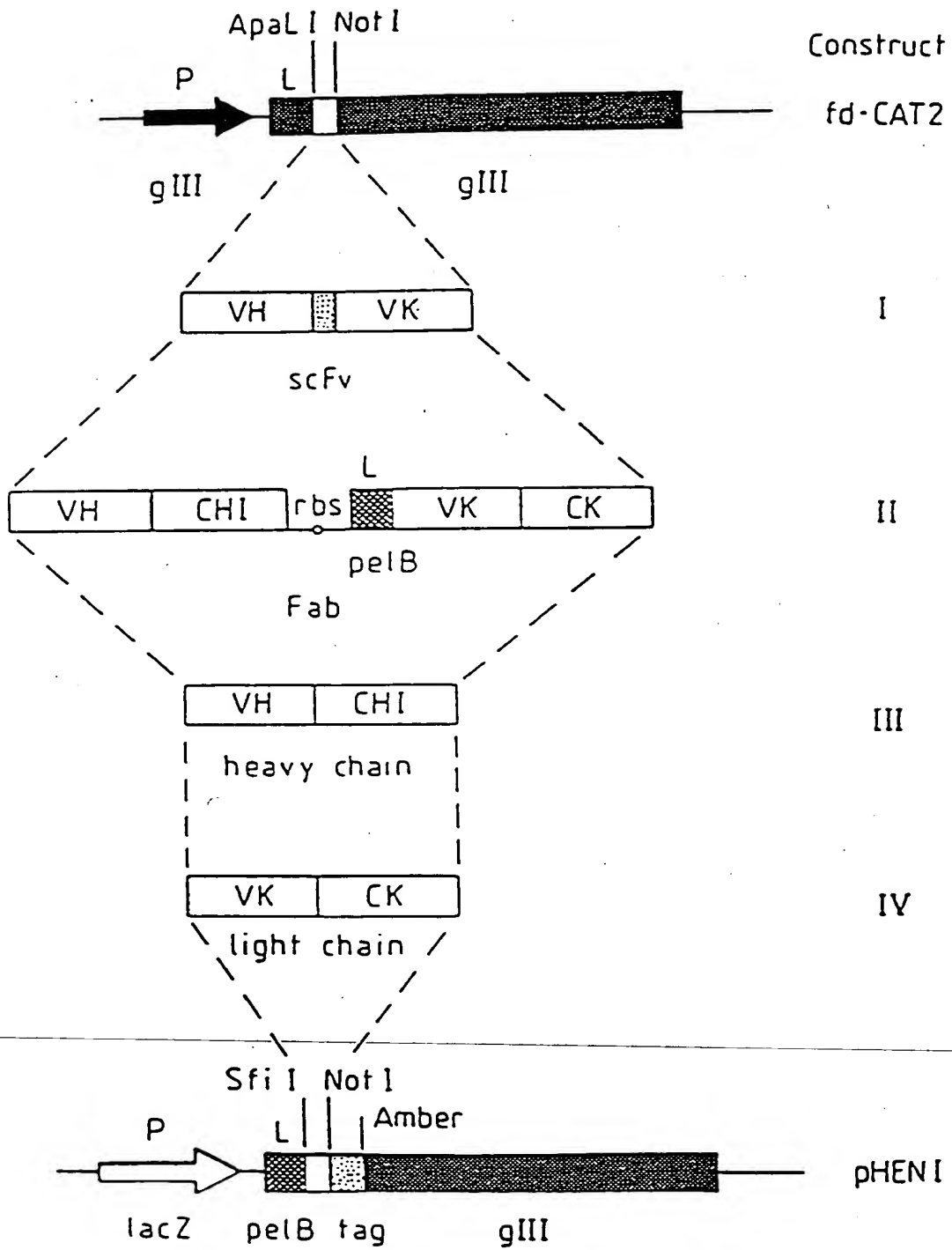
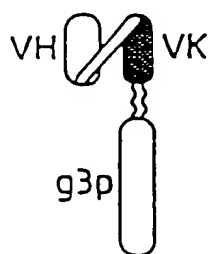
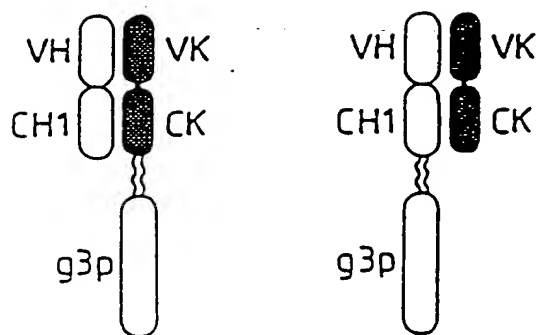


Fig.28.

Fab



scFv

Fig.29.

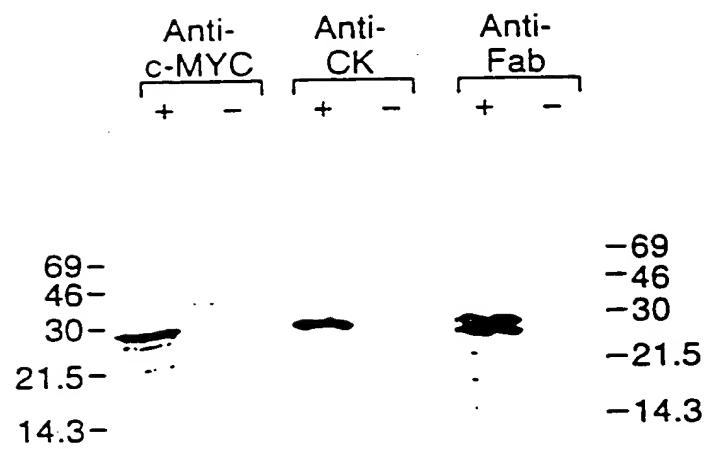


Fig.30.

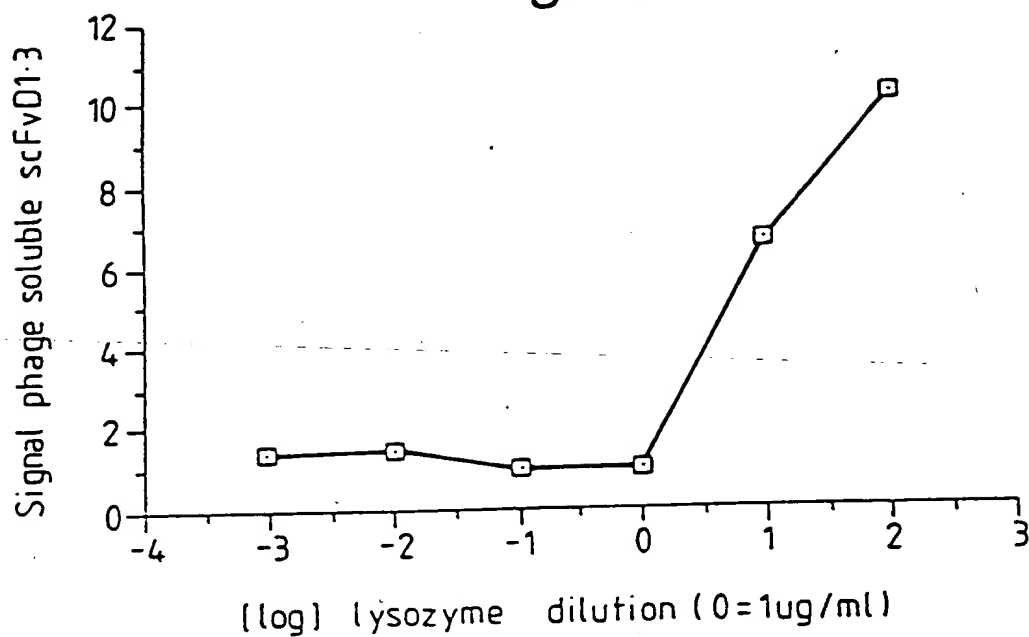


Fig.31.

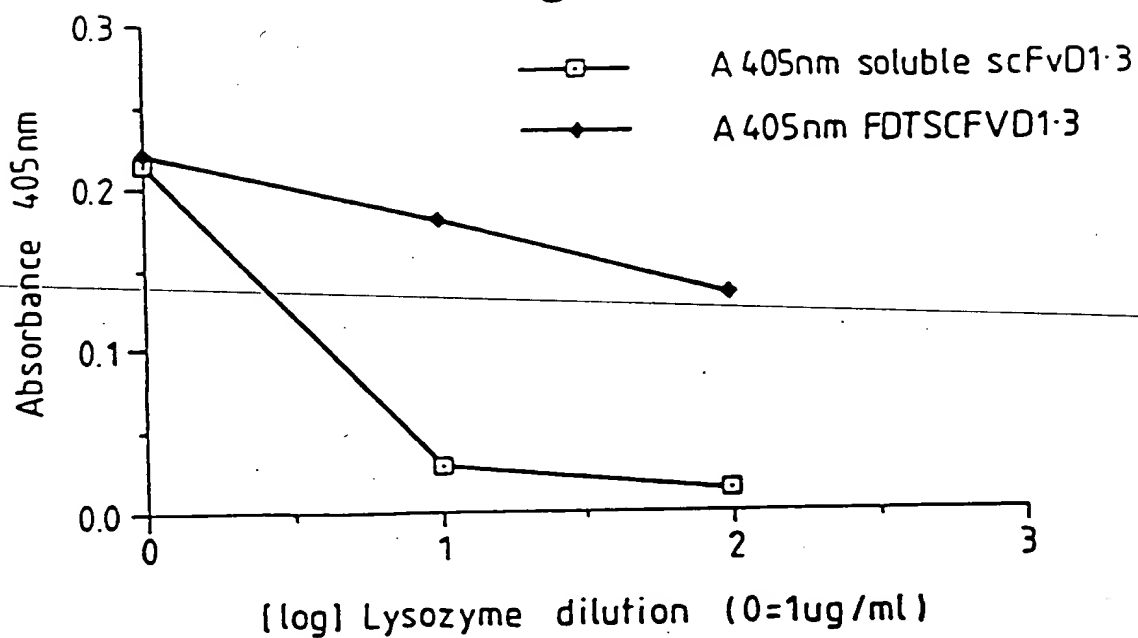


Fig.32.

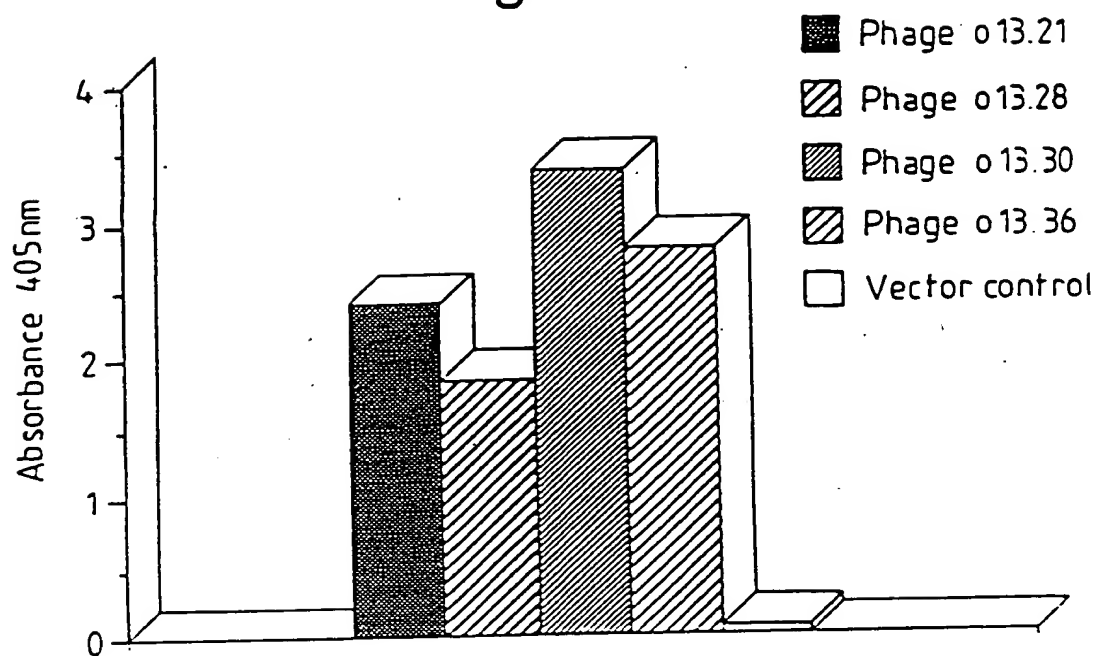


Fig.33.

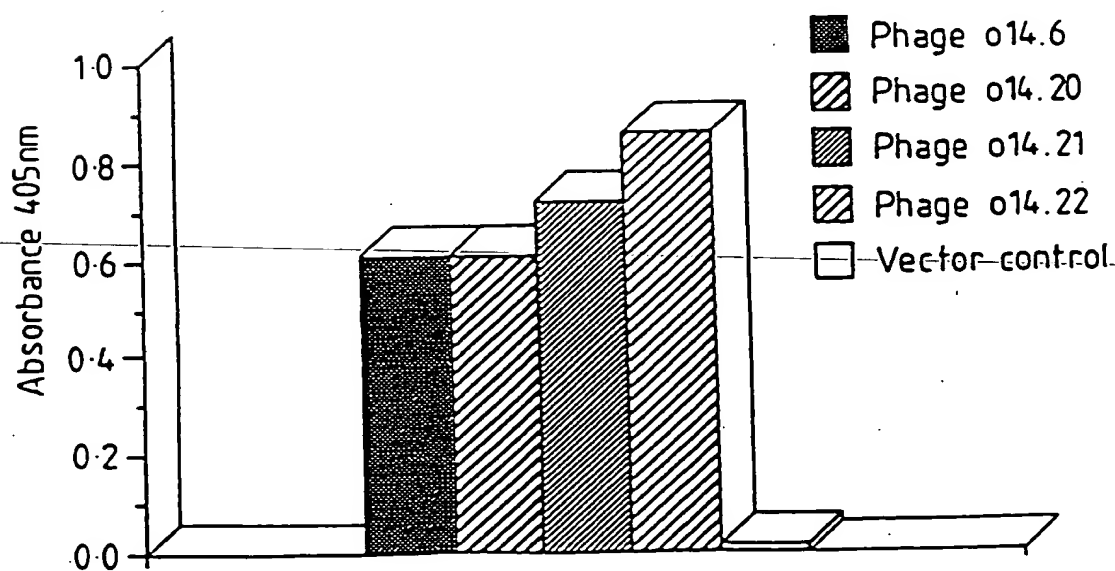


Fig.34.

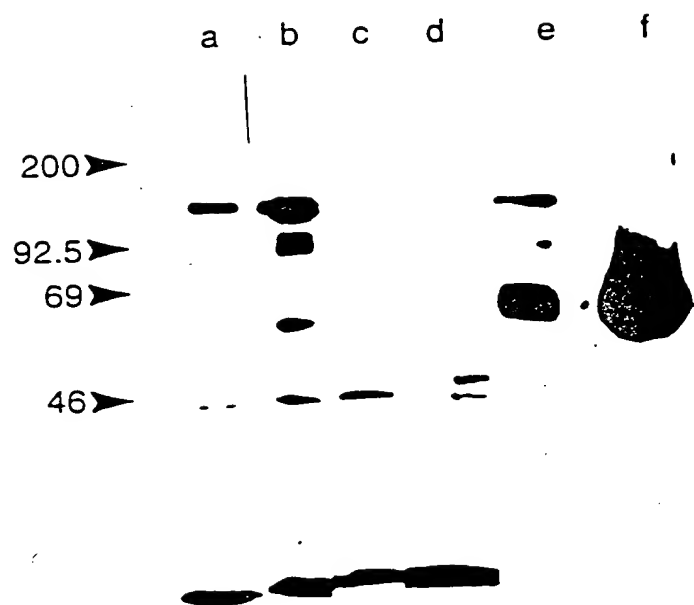


Fig.35A.

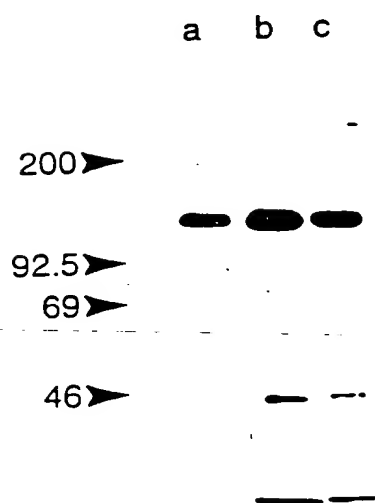


Fig.35B.

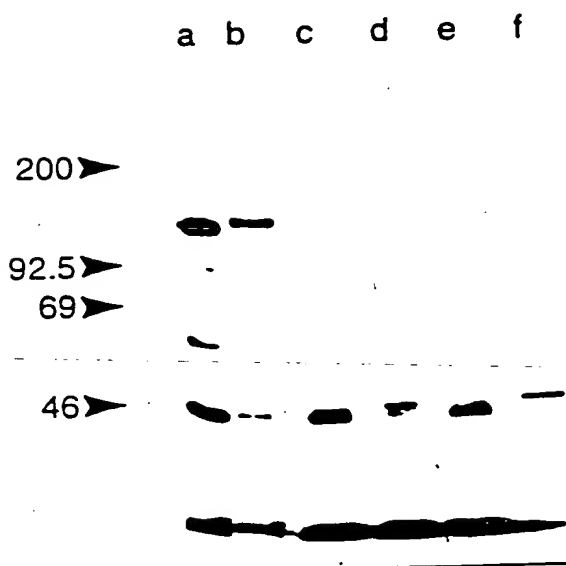


Fig.36.

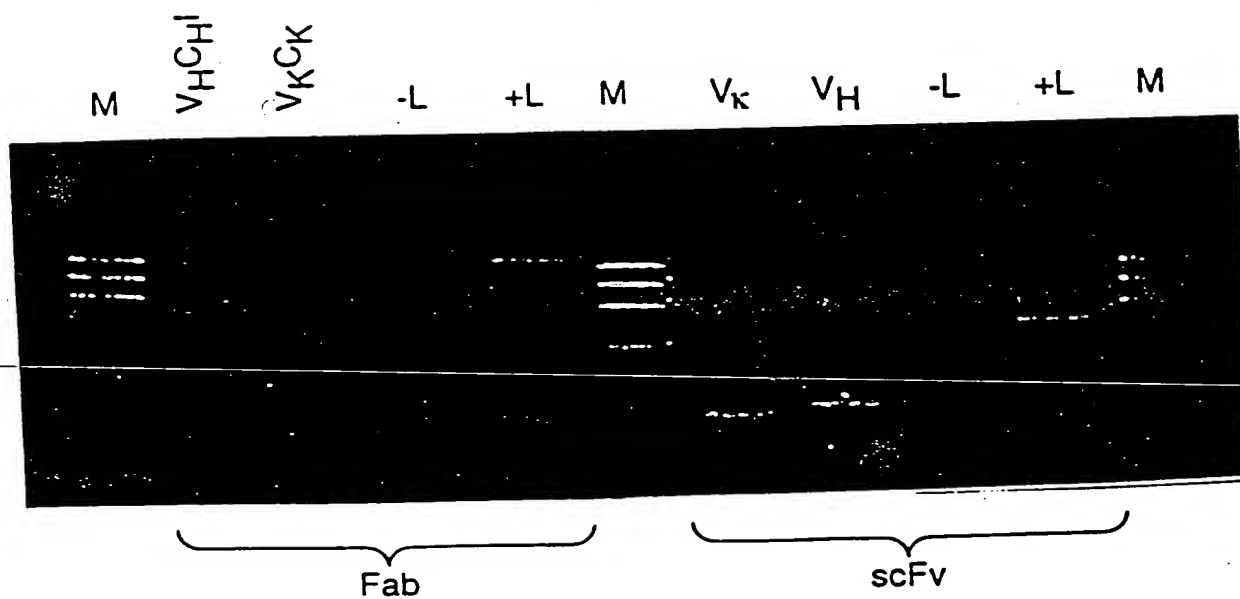


Fig.37.

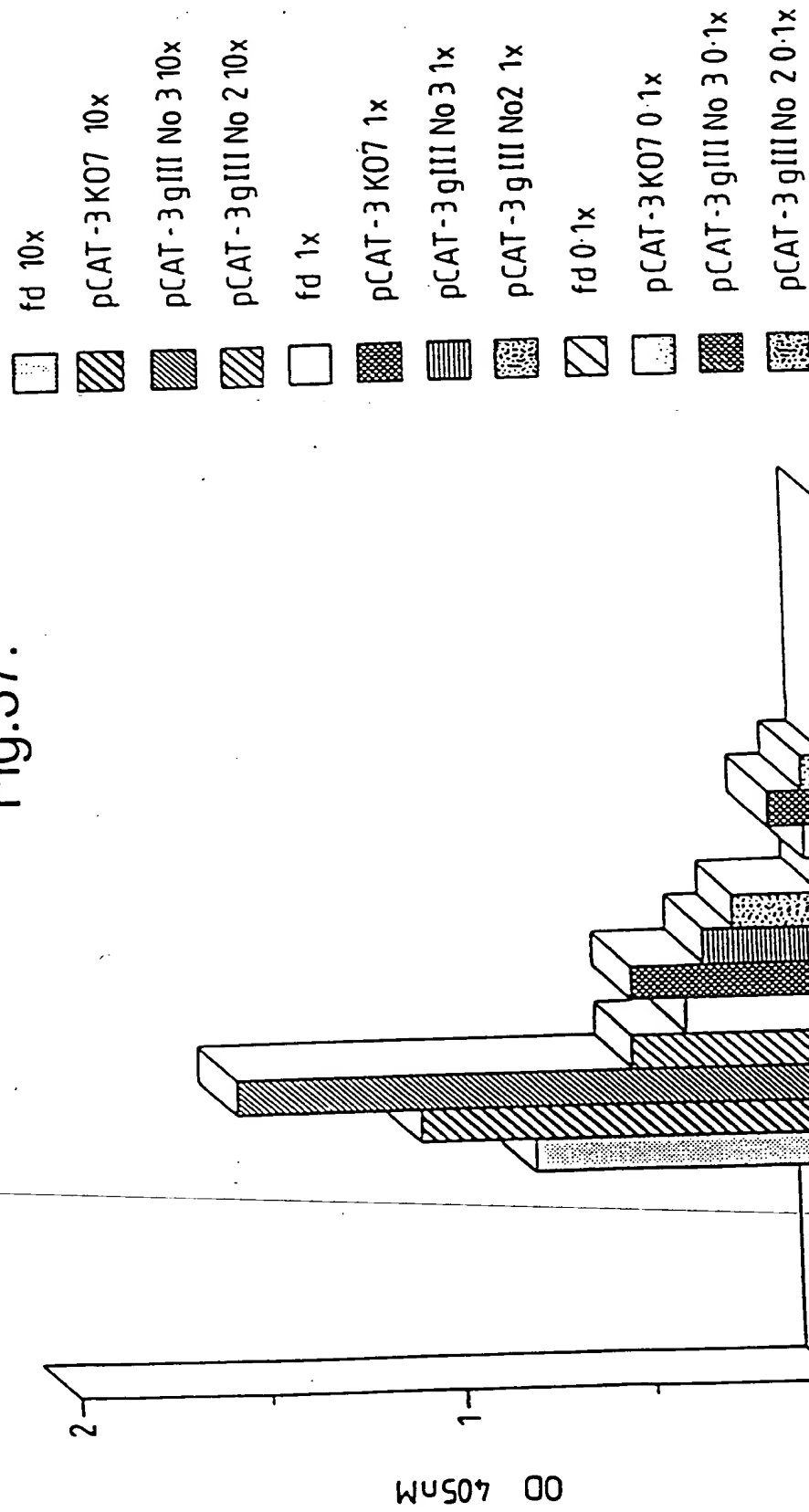


Fig.38A.



Fig.38B.

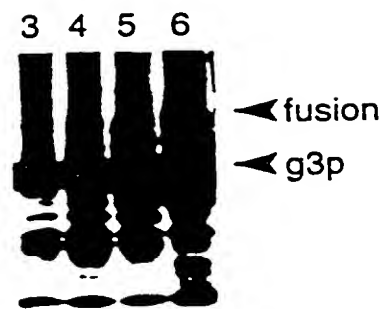


Fig.39.

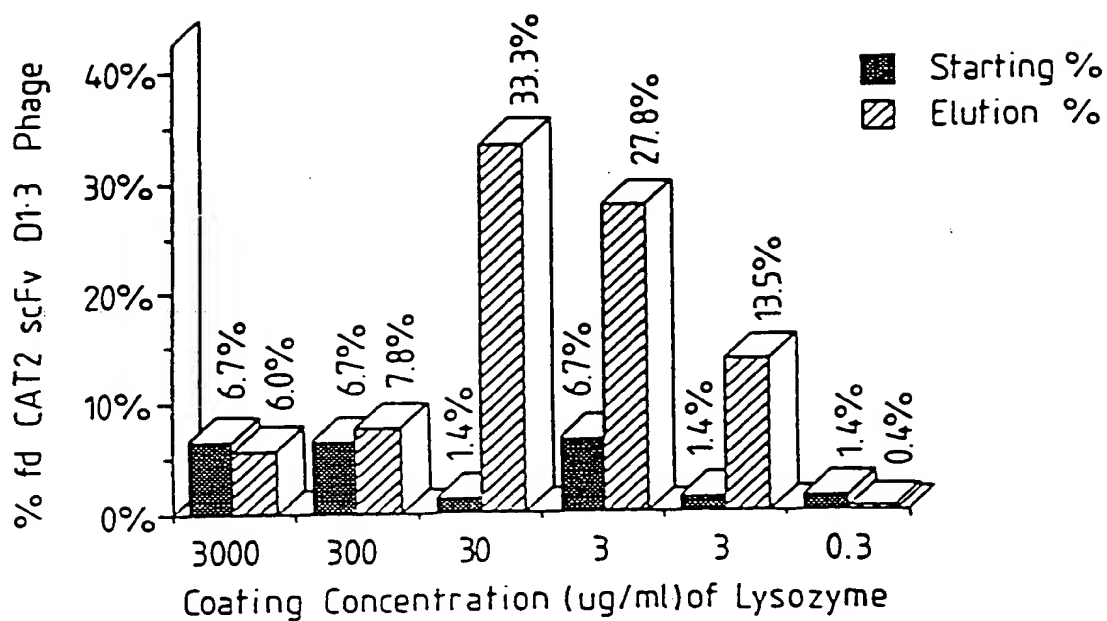


Fig.40.

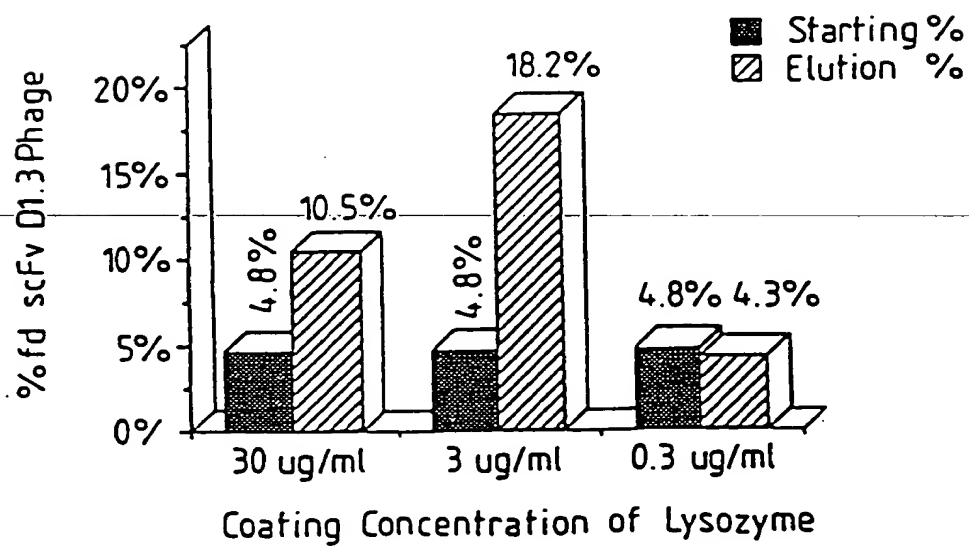


Fig.41.

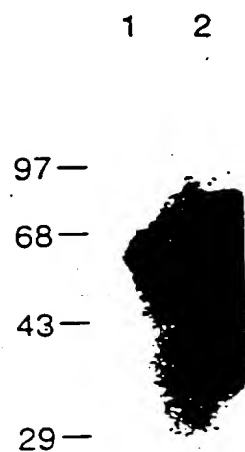


Fig.42.

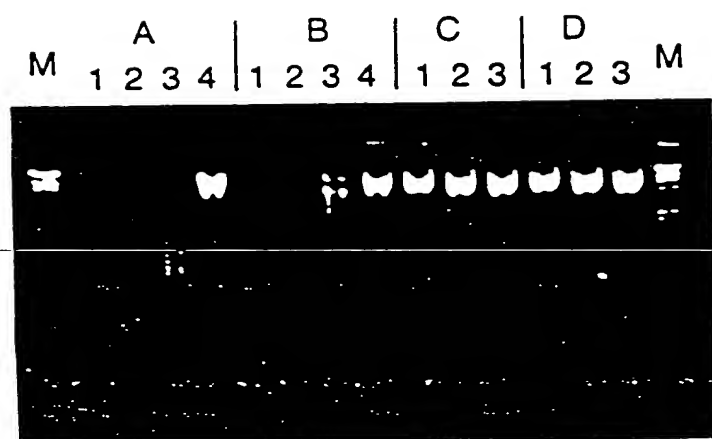


Fig.43.

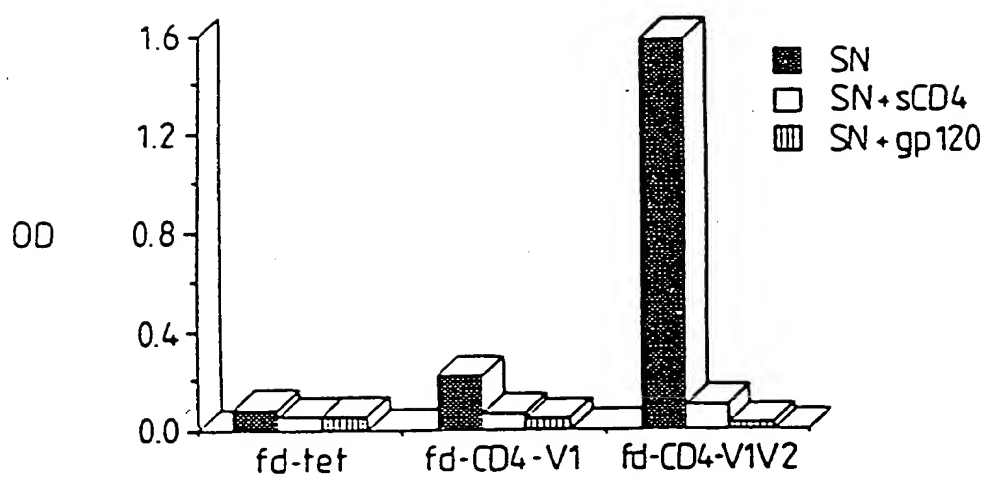


Fig.44 (i).

10 20 30 40 50 60 70 80 90
 TTCTATTCTACAGTGCNAGGTCACAGCTGCAGCAGTCTGGGGCTGAGCTTGTGAAGCCTGGGGCTTCAGTGAAGCTGTCTCTGCAAGGCT
 AAGATAAGAGTGTACAGTGTCCAGGTCGACGTCGTCAGACCCCGACTCGAACAACATTCCGACCCCGAAGTCACATTTCGACAGGACGTTCCGA
 pheTyrSerHisSerAlaGlnValGlnLeuGlnSerGlyAlaGluLeuValLysProGlyAlaSerValLysLeuSerCysLysAla
 100 110 120 130 140 150 160 170 180
 TCTGGCTACACCTTCACACGCTACTGGATGCACCTGGGTGAAGCAGAGGCCCTGGACGAGGCCCTTGAGTGGATTGGAGGATTGATCCTTAAT
 AGACCGATGTGGAGTGTGATGACCTACGTGACCCACTTCGTCTCCGGACCTGCTCCGGAACCTCACCTAACCTTCCCTAACCTAGGATTA
 SerGlyTyrThrPheThrSerTyrTrpMetHisTrpValLysGlnArgProGlyArgGlyLeuGluTrpIleGlyArgIleAspProAsn
 190 200 210 220 230 240 250 260 270
 AGTGGTGGTACTAAGTACANTGAGNAGTTCAGAGAGCAGAGCCACACTGACTGTAGACNAACCCCTCCAGCACAGCCCTACATGCAGCTCAGC
 TCACCACCATGATTCATGTTACTCTTCAAGTCTCTCGTTCGGGTGACTGACATCTGTTGGGAGGTCGTGTCGGATGTACGTCGAGTCG
 SerGlyGlyThrLysTyrAsnGluLysPheLysSerLysAlaThrLeuThrValAspLysProSerSerThrAlaTyrMetGlnLeuSer
 280 290 300 310 320 330 340 350 360
 AGCCTGACATCTGAGGACTCTGCGGTCTATTATTGTGCAAGNTACGACTACGGTAGTACTACTTTGACTACTGCGGCCCAAGGGACC
 TCGGACTGTAGACTCCTGAGACGCCAGATAATAACACGTTCTATGCTGATGCCATCATCGATGATGAACCTGATGACCCCGGTTCCCTGG
 SerLeuThrSerGluAspSerAlaValTyrTyrCysAlaArgTyrAspTyrGlySerSerTyrTyrPheAspTyrTrpGlyGlnGlyThr
 370 380 390 400 410 420 430 440 450
 ACGGTCACCGTCTCCTCNGGTGGAGCGGTTTCAGGCGGAGGTGGCTCTGGCGGTGGCGGATCCAGGCTGTTGGGACACAGGAATCTGCA
 TGCCAGTGGCAGAGGAGTCCACCTCCGCCAAGTCCGCCCTCCACCGAGACCGCCACCGCTAGGTCGACACACCCCTGTGTCTCTTAGACGT
 ThrValThrValSerSerGlyGlyGlySerGlyGlyGlySerGlyGlyGlySerGlnAlaValGlyThrGlnGluSerAla
 460 470 480 490 500 510 520 530 540
 CTCACACATCACCTGGTGAACAGTCACACTCACTTGTGCTCAAGTACTGGGGCTGTTACAACTAGTAACCTATGCCAACTGGGTCCAA
 GAGTGGTGTAGTGGACCACTTTGTGAGTGTGAGTGAACAGCGAGTTTCATGACCCCGACAAATGTTGATCATGATACGGTTGACCCAGGTT
 LeuThrThrSerProGlyGluThrValThrLeuThrCysArgSerSerThrGlyAlaValThrThrSerAsnTyrAlaAsnTrpValGln
 550 560 570 580 590 600 610 620 630
 GAAAACACGATCATTTTACCTGGTCTAATAGGTGGTACCAACAACCGAGCTCCAGGTGTTCTCGCCAGATTCTCAGGCTCCCTGATT
 CTTTGTGCTAGTAAATAAGTGACCAGATTATCCACCATGTTGTTGCTCGAGGTCACAGGACCGTCTAAGAGTCCGAGGGACTAA
 GluLysProAspHisLeuPheThrGlyLeuIleGlyGlyThrAsnAsnArgAlaProGlyValProAlaArgPheSerGlySerLeuIle

Fig.44 (ii).

640	650	660	670	680	690	700	C	G	710	720
GGAGACAAGGCTGCCCCC	TCACACAGGGGCACAGACTGAGGATGAGGC	AAATATATTTCTGTGCTCTATGG	AC	AGCAACCAAT	GGGTG					
CCTCTGTTCCGACGGGAGTGGTAGTGTCCCCCGTGTCTGACTCCTACTCCGTTATATAAGACACGAGATACCA	TGTGTGCTCGTTGGTAACCCAC									
GlyAspLysAlaAlaLeuThrIleThrGlyAlaGlnThrGluAspGluAlaIleTyrPheCysAlaLeuTrp	TyrPheCysAlaLeuTrp									
730	740	750	760	770						
TTCGGTGGAGGAA	CAAACTGACTGTCTCGAGATCAAAACGGGGCGCCGC									
AAGCCACCTCCTTGGTTGACTGACAGGAGCTCTAGTTGCCCCCGCGCG										
PheGlyGlyThrLysLeuThrValLeuGluIleLysArgAlaAla										

(SEQ ID NO. 261)

(SEQ ID NO. 262)

Fig.45.

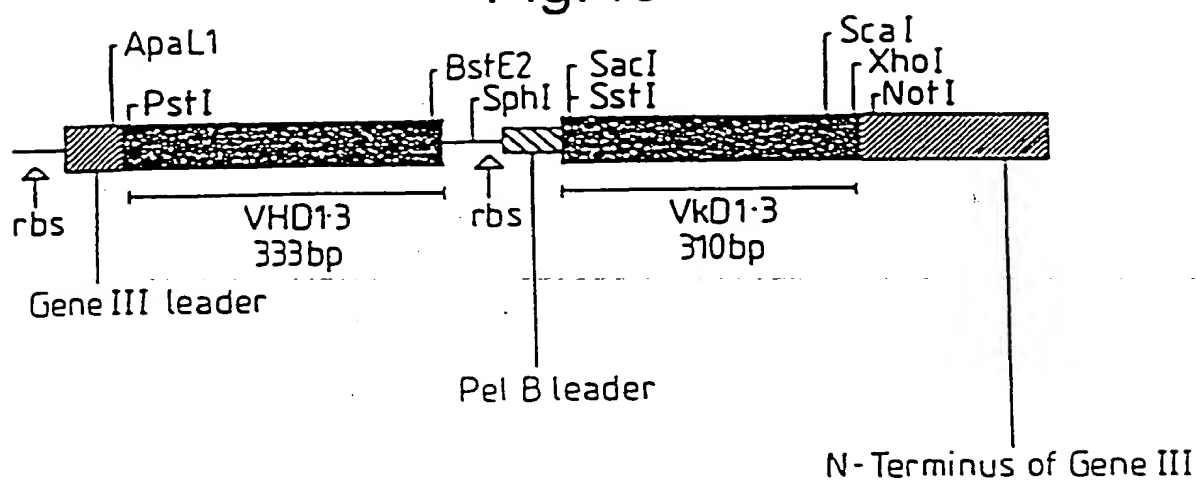


Fig.46.

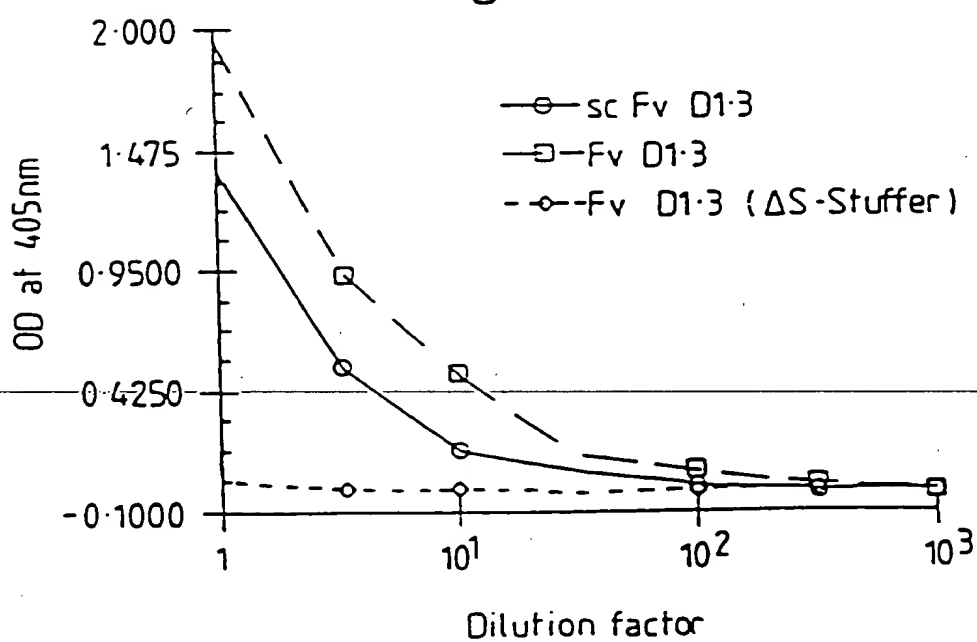


Fig.47.

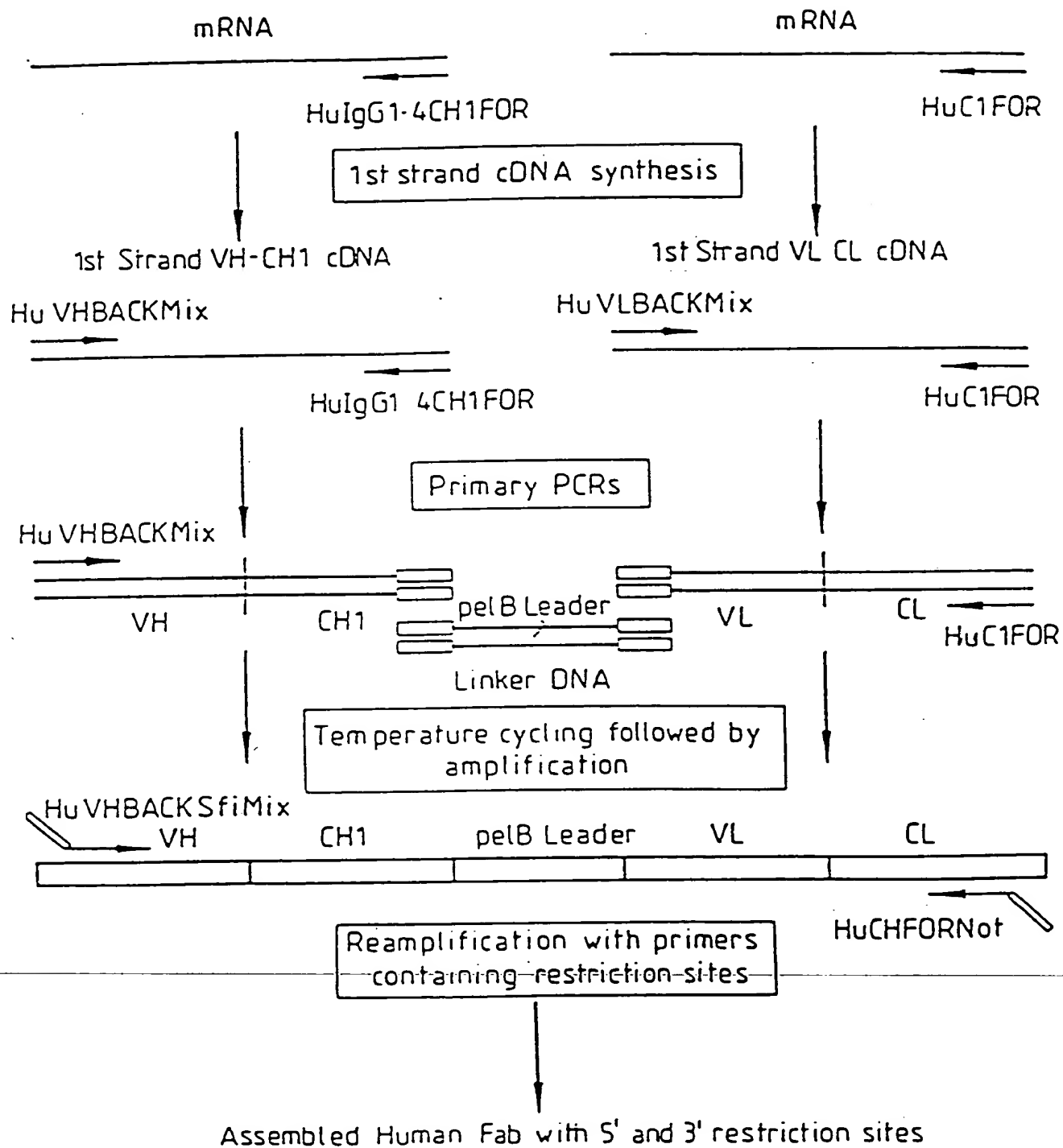


Fig.48(i)

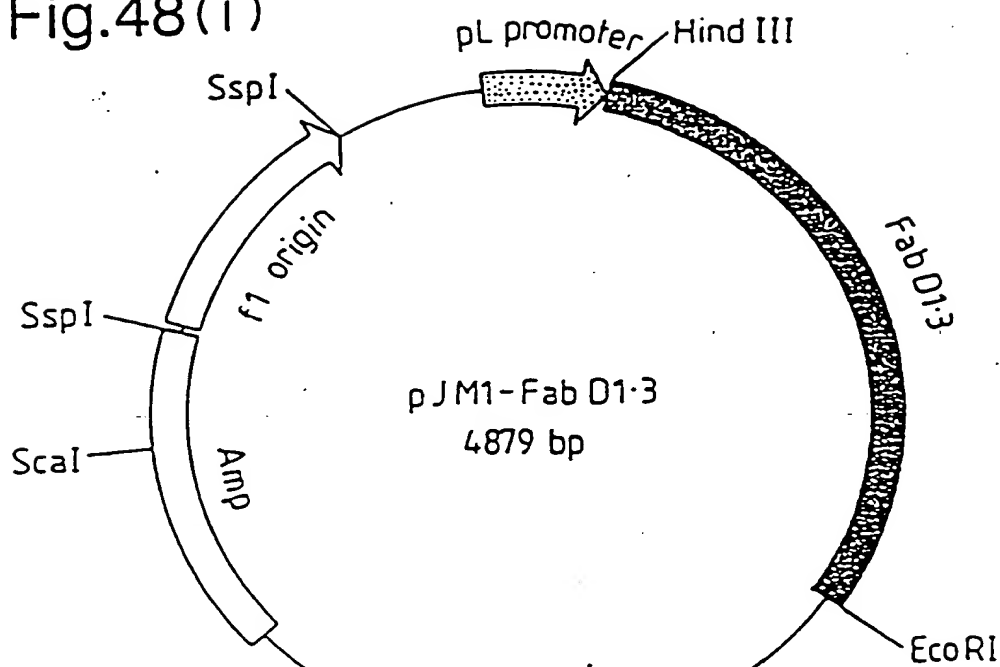


Fig.48(ii)

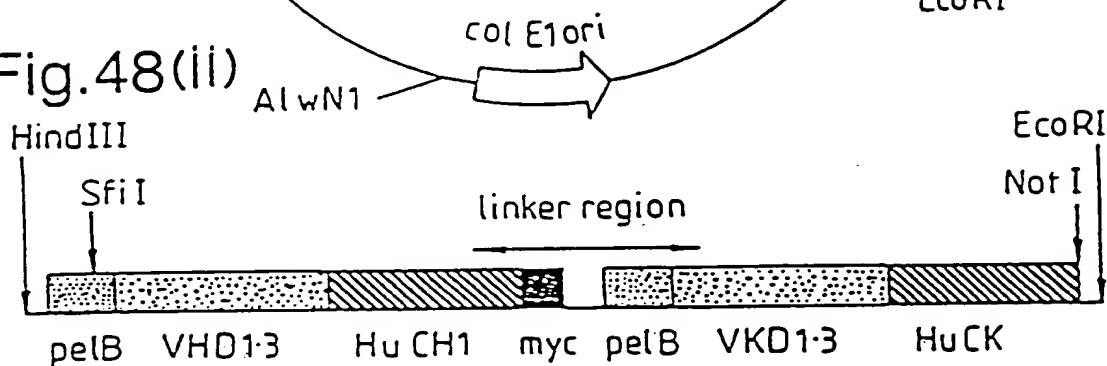


Fig.48(iii)

← 3' Human CH1 and hinge →
 K P S N T K V D K K V E P K S S T K T H T
 AACCCACGCAACACCAAGGTCGACAAGAAAGTTGAGCCCAATCTTCAACTAAGACGCACACA

→ myc peptide tag →
 S G G E Q K L I S E E D L N * *
 TCAGGAGGTGAACAGAAGCTCATCTCAGAGAGGATCTGAATTAATAAGGGAGCTTGCAATGCA

(SEQ ID NO. 263)

← pelB leader →
 M K Y L L P T A A A G L
 AATTCATTTCAAGGAGACAGTCATAATGAATACTATTGCTACGGCAGCCGCTGGATTGT

→ 5' Vk →
 L L P A A Q P A M A D I E L T Q S P
 TATTAAGTCTGCTGACCAACAGGATGGGACATGAGTTCAACCCAGTCTCC

(SEQ ID NO. 264)

(SEQ ID NO. 265)

Fig.49.

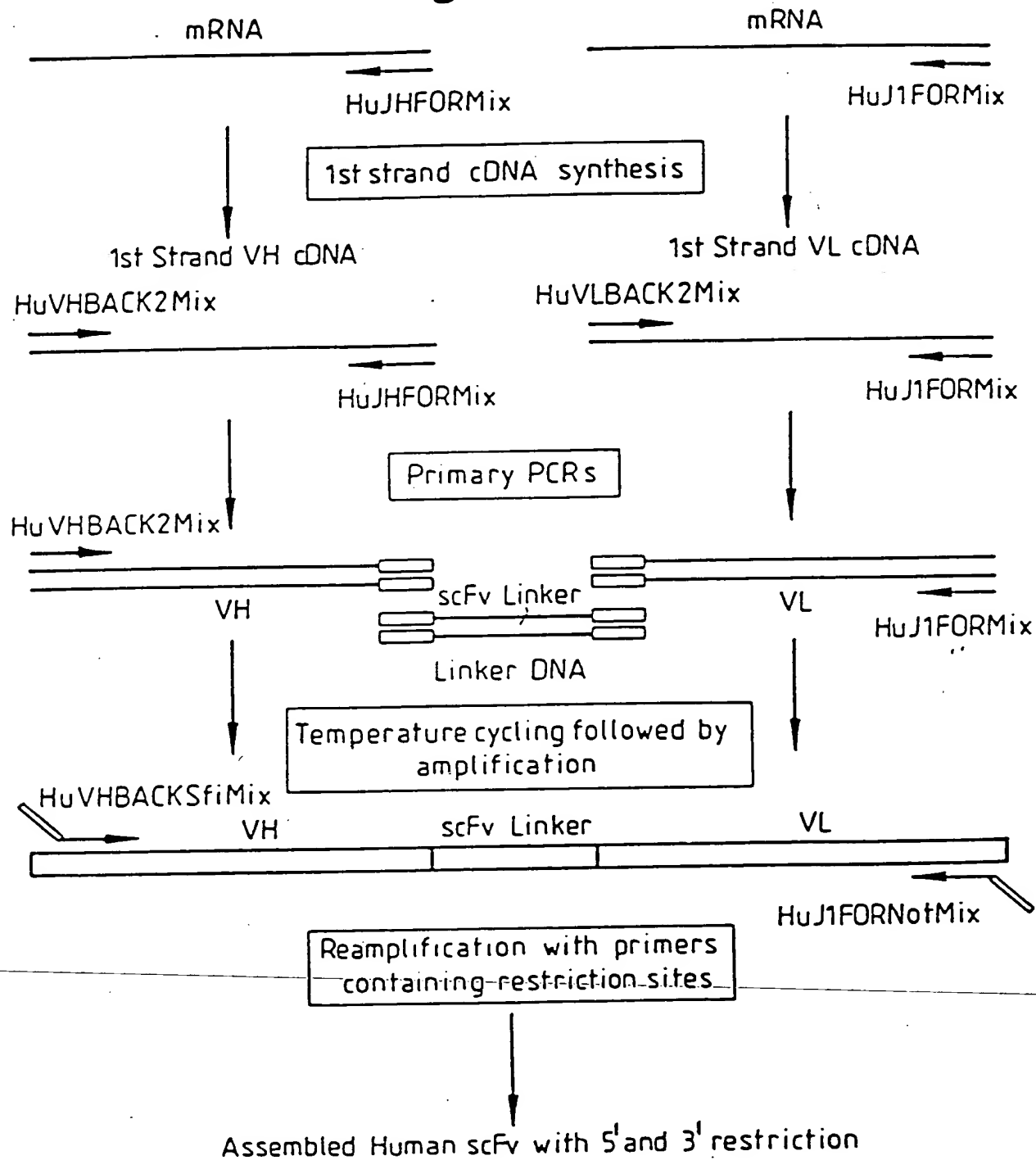


Fig.50(i)

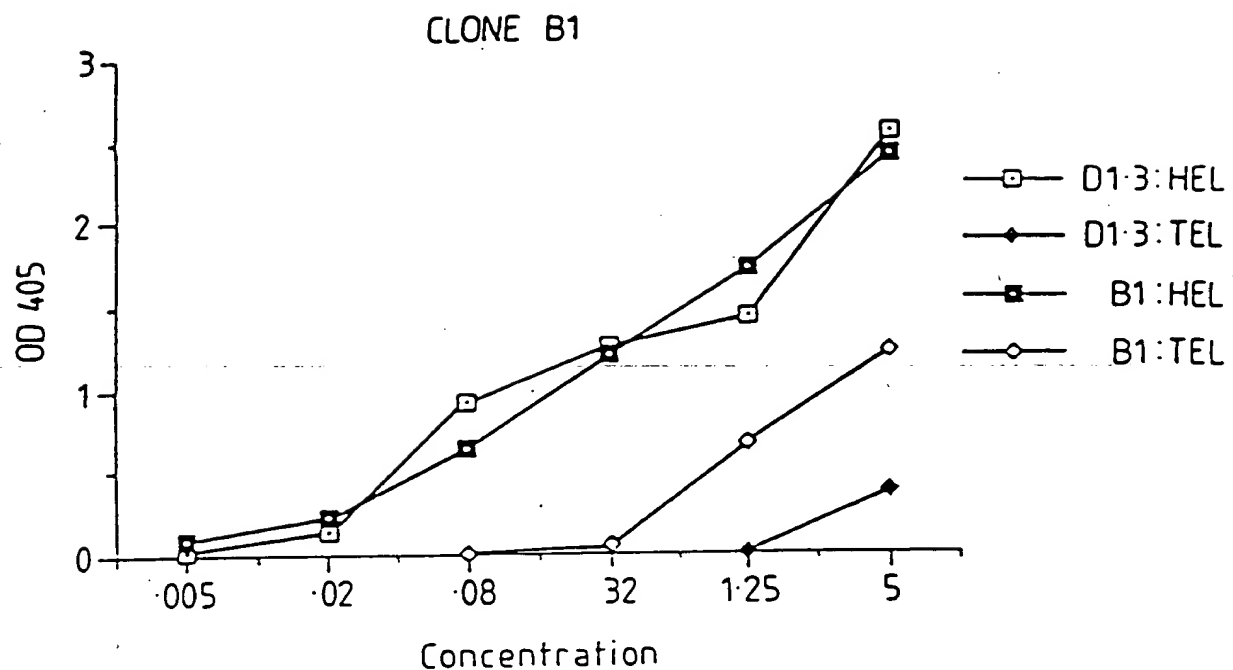


Fig.50(ii)

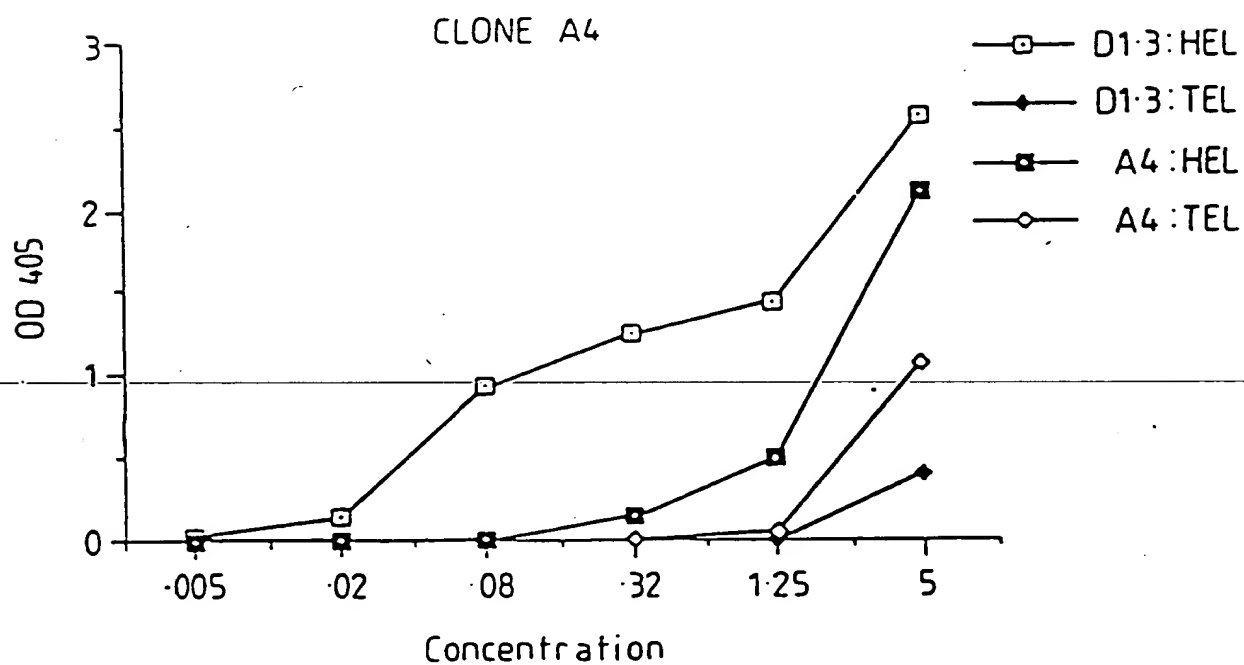


Fig.51.

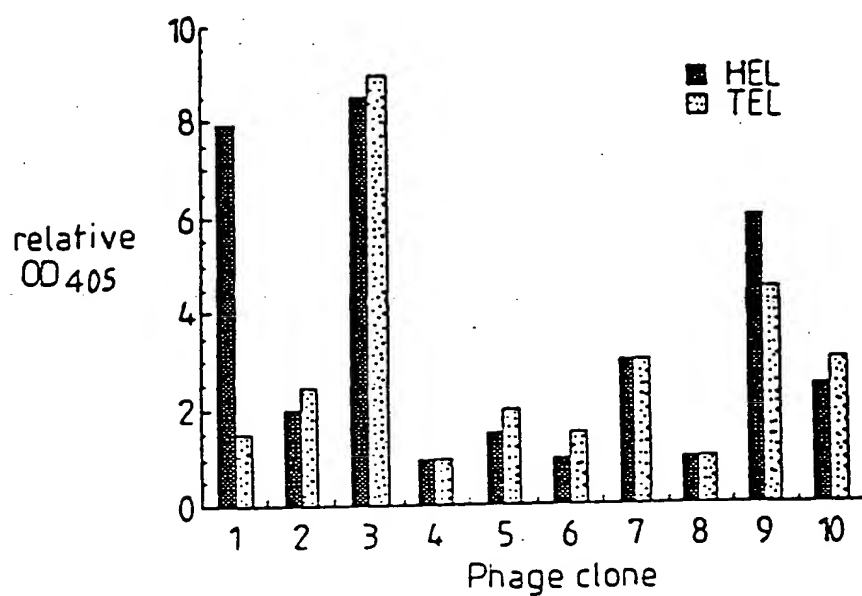


Fig.53.

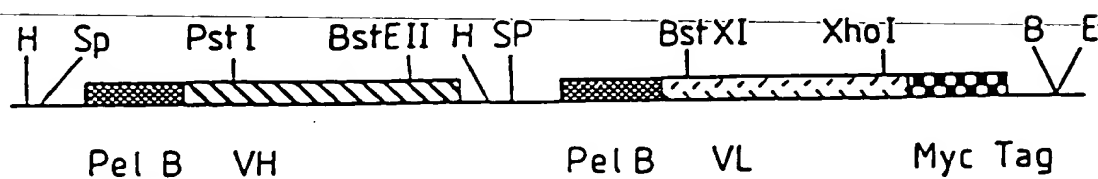


Fig.52.

	CDR 1	CDR 2
D1.3	DIQMTQSPASLSASVGETVTITCRASGNIHNYLA	WYQQKQCKSPQLLVYYTTLAD
M1F	DIELTQSPSSLASLGERVSLTCRASQDIGSSLN	WLQQEPDGTIKRLIYATSSLDS
M2I	DIELTQSPALMAASPGEKVITICSVSSSISSNLHWYQQKSETSPKPIYGTSNLAS	

	CDR 3	
D1.3	GVPSRFSGSGGTQYSLKINSLQPEDFGSYQCQHFWSPTPTFGGGTKLEIKR	(SEQ ID NO. 266)
M1F	GVPKRFGSRGSDYSLTISSLESEDFVDYCLQYASSPWTFGGGTKLELKR	(SEQ ID NO. 267)
M2I	GVPVRFSGSGGTSLTISSMEAEDAATYYCQQWSSYPITFGAGTKLEIKR	(SEQ ID NO. 268)